AN INTRODUCTION TO BIOPHYSICS

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WITH A FOREWORD BY

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SECOND EDITION

WITH 116 ILLUSTRATIONS

LONDON

J. & A. CHURCHILL

40 GLOUCESTER PLACE

PORTMAN SQUARE

1929

TO

I. R. V. M.

Printed in Great Britain

PREFACE TO THE SECOND EDITION

THE scope of the book has been slightly altered to make it more in accord with the *Syllabus of Biophysics* suggested by the *General Medical Council.* Sections I. and II., with the corresponding exercises in Part II., cover the syllabus of the *Physical Physiology* required by *The Examining Board in England of the Royal College of Physicians of London and the Royal College of Surgeons of England.* The text, however, has not been cut merely to suit examinations, but an attempt has been made to view the human body as far as possible as a physical machine. To do this adequately a knowledge of mathematics beyond the stage usually professed by medical and other students of the Biological Sciences is necessary. We have therefore cut down mathematical treatment to the minimum and have indicated where the student who desires to study the subject further may get additional information.

In spite of efforts to keep the book reasonably small, expansion has taken place. A new chapter on Emulsions and Soaps has been added, and the chapters on Surface Tension, General Receptors, Eye, Ear, Voice and Movements of the Limbs have been almost entirely rewritten. The greatest changes have been made in Part II., as the result of six years' teaching experience.

The main purpose of this preface is to record my thanks to all who have helped me in the revision. My Staff at the College of Medicine has generously come to my aid. Messrs. Secker and Saunders have read all the proofs and made many valuable suggestions. Mr. Saunders and my wife have checked most of the calculations. I am also indebted to my wife for much help in that most tiring of tasks, the compilation of the index of subjects.

Illustrations have been drawn from various sources, and I desire to thank the various authors who have given me permission to use their blocks. Especial mention should be made of the permission freely given by Professor L. J. Henderson to use his "Alignment Chart," and by Professor Leathes for his series of "Myelin" figures.

Figs. 61, 75, 80, 81, 99 and 100 are from the late Professor Starling's *Principles of Human Physiology;* 2, 21, 24 and 25 from Pryde's *Recent Advances in Biochemistry;* 68 and 83 from Lovatt Evans' *Recent Advances in Physiology;* 72 from Goulden's *Refrac-*

tion of the Eye; 5 and 12 from Crocker and Matthew's *Theoretical and Experimental Physical Chemistry.* To all these authors and to their publishers, Messrs. J. and A. Churchill, I desire to express my thanks.

I am indebted to Messrs. Baird and Tatlock, London, for the loan of the blocks for Figs. 8, 18, 22, 45, 53, 67, 104, 106 and 109.

I wish to record my thanks to Miss E. M. Paul, of the King Edward School of Art, Armstrong College, for the original drawing for Fig. 66, and to Mr. J. Robson for the rough drawings from which all the other new illustrations were made.

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PREFACE TO THE FIRST EDITION

THIS book makes no pretensions to be a complete or even a systematic survey of Biophysics. Its object is partly to be explanatory. Current medical publications are full of terms culled from physico-chemical and physical terminology; the clinician of to-day clothes his ideas in words unknown to his brethren of yesterday; his phraseology, at least, is physical.

Apart from and beyond a mere explanation of physico-chemical terms, an attempt has been made in the following pages to present physiological phenomena from a purely physical standpoint. The problems of life, and vertebrate life in particular, have been viewed through a physicist's eyes. This does not necessarily imply that the matter of the book is permeated with mechanistic philosophy. We are all, more or less, amateur philosophers, but we would be poor scientists indeed if our " views " were permitted to colour our facts. Phenomena, as they appear to-day, are set out for the critical examination of the student. " He will have all the facts and circumstances fully mobilised, standing up side by side before him like an awkward squad, and there is nothing more awkward than some facts that have to stand out squarely in the daylight! And he inquires into their ancestry, makes them hold out their tongues, and pokes them once or twice in the ribs, to make sure that they are lively and robust facts capable of making a good fight for their lives. He never likes to see one thing too large. . . . lest he sees something else too small; but will have everything in true proportion." (David Grayson.)

It is a great pleasure to me, on reading over the final proofs, to notice how generously my masters and colleagues have come to my aid. Quite apart from the direct help given me by Professors Noël Paton and E. P. Cathcart, who contribute the opening and closing chapters of the theoretical part of the book, I have received daily encouragement from them in my task, for which I express my sincere gratitude. If this effort to make plain the essentials of Biophysics is in any way successful it is due to the truly scientific atmosphere of the Institute of Physiology which they govern and inspire.

I beg to record my obligation to Dr. Shanks for the care he has devoted to the chapter on the eye; to Dr. Morris for reading the

first three sections of the book in slip-proof; to Dr. Watt, Lecturer on Psychology in this Institute, for reading the chapters on Receptors and for his suggestions thereon; to Dr. Wishart, because, by reading many of the proofs and by checking mathematical matter, he has saved me from many a fault and blunder.

My debts to previous authors are many and I cannot own them all. Discerning readers will see, for example, the ideas of my old teacher, Professor Soddy, mirrored in certain of the earlier chapters; Professor Thompson's *Growth and Form* is the basis of part of Chapters XVI., XXIV. and XXXIV.; McKendrick, Gray, Wrightson, Keith, and Watt are the sources from which much of Chapters XIX. and XXIX. have been drawn. A book of this nature could not be written without constant reference to the *Principles of General Physiology*. If my *Introduction* but serves to turn some student to the great book of Professor Bayliss, to meet the master mind, it will have succeeded.

I am under obligation to the authors and publishers of several books from which illustrations have been borrowed.

To Professor Noël Paton and Messrs. Green for permission to use eight figures from the *Essentials of Human Physiology* (viz., Figs. 41, 47, 85, 86, 95, 98, 101 and 116); to Professor Starling and Messrs. Churchill for the following figures from *Principles of Human Physiology:* 1, 10, 16, 31, 56–59, 63, 65, 73, 96, 97, 103, 105, 107, 112 and 114; to Mr. Crowther for Fig. 39 from *Molecular Physics*, and to Mr. Emil Hatschek for Figs. 15, 19 and 20 taken from *An Introduction to the Physics and Chemistry of Colloids*, both books from Messrs. Churchill.

To Professor Cushny for leave to reproduce the ideal diagram of a Malpighian corpuscle (Fig. 46) from his monograph on *The Secretion of Urine* (Messrs. Longmans, Green and Co.); to Professor Soddy and "The Electrician" Publishing Co., for the diagram of the gold-leaf electroscope (Fig. 40) from *Radioactivity*.

To Dr. Bradford for allowing me to reproduce, from the *Biochemical Journal*, his photograph of adsorptive stratification (Fig. 17), and to Professor Roaf for the $p\mathrm{H}$–$\mathrm{C_H}$ graph reproduced from the Proceedings of the Physiological Society (Fig. 115).

To Messrs. the Cambridge and Paul Scientific Instrument Co. for the figures illustrating the electro-cardiograph (Figs. 91, 92 and 93); to Messrs. Hawksley for those of the viscosimeter (Figs. 108 and 110), and to Messrs. Gallenkamp for Figs. 3 and 102 of the bomb calorimeter.

The remaining illustrations were drawn by Dr. G. M. Wishart, Assistant in the Department of Chemical Physiology, and by Mr.

John Waters, a student of medicine here. To their skill and care I owe much.

I am greatly obliged to Mr. A. V. Steen, B.Sc., one of our demonstrators, for reading all the proofs. The freedom of the matter from certain errors is the result of his painstaking efforts.

Finally, I desire to record my gratitude to my publishers for their patience and courtesy during the prolonged period of publication and to the printers for the care they have taken and the consideration shown when my ignorance made large demands on their time and patience.

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INTRODUCTION

BY PROFESSOR D. NOËL PATON, M.D., LL.D., F.R.S.

ON looking back over a forty years' association with physiology nothing is more striking than the influence which the application of physics has exercised upon the progress of the sciences.

I well remember that, as long ago as 1878, my first teacher began his lectures on the Institutes of Medicine by defining physiology as the application of physics and chemistry to the study of the body in action.

But at that time the possibility of applying these sciences was limited. In the first place, their development, and especially the development of physics, was not sufficiently advanced. The dissociation of atoms into ions was hardly recognised, the significance of Graham's colloids was not appreciated, and the phenomena of surface tension had hardly been applied to molecular physics. In the second place, physiologists were then generally men trained for medicine, whose education in physics and chemistry had been extremely limited. Of course, there were notable exceptions—*e.g.* Helmholtz and du Bois Reymond.

These older physiologists had to be content with recording phenomena rather than with explaining them, and they loved to chronicle their observations in high-sounding Greek names. Can one ever forget the sense of profound knowledge which one enjoyed as a junior student in mastering such terms as "delomorphous" and "adelomorphous" as descriptive of the cells of the stomach? The so-called chemical physiologists were perhaps the worst offenders. For, having isolated, or thought they had isolated, some constituent of the body of quite unknown chemical constitution, they promptly gave it a name with no connection with its chemical nature, and these names have generally continued in use, to the confusion of generations of students. In the present age of "hormones" and "vitamines" one wonders how far the tendency has been eradicated.

It was the "what happens?" which interested these older workers: "Why it happens?" was generally beyond them, and vague theories of some peculiar and special vital action took the place of actual demonstration.

Undoubtedly the association of physiology with the more exact

science of physics, based as it is so largely upon mathematics, has had an enormous effect in getting rid of this habit of vague theorising and has materially helped to clarify minds " debauched with the so-called science of biology " as Tait, in the early 'eighties, was wont to describe our mental condition.

It has also stimulated the critical faculty, which insists upon a clear proof and demonstration as a basis of conclusions.

It is much to be regretted that even at the present time the importance of a mathematical training, so essential for the study of physics, is not generally recognised, and that it is still possible to take a higher degree in science without this necessary preparation.

It has been through the co-operation of physics and chemistry that the solution of many of the problems of life have been reached, and as the possibility of reaching these solutions has become more generally recognised, the spirit of scientific curiosity —the desire to know, which is the basis of all scientific work— has been stimulated; although probably in the future, as in the past, humanity will still be divided into the enormously large class of those who have no real desire to understand the workings of nature and the very small class of those who have the spirit of curiosity, who do desire to know. These alone are the scientists, although many science graduates belong to the major class.

With or without any wider diffusion of the spirit of curiosity, the development of the critical faculty and the better training of the younger workers in physics and chemistry has brought physiology nearer to the position of an exact science, and with this, its value as a training for students of medicine has greatly increased. The doctor, in making a diagnosis, has not merely to observe and record what has happened, but he must ascertain why it has happened. His problems are the same in nature and his methods are the same as those of the physiologist, and thus physiology has regained its position as the Institutes of Medicine.

The doctor of the past considered that he had made a diagnosis when he was able to give his patient's disease a name. The physician of the future will care less and less for such names. He will simply be concerned with the solution of the questions— " What is abnormal ? " " Why is it abnormal ? " Perhaps it is wiser not to enquire too curiously into the position of the physician of the present.

The development of physiology on the lines indicated has also made possible the growth of the sciences of experimental pathology, of experimental medicine and of pharmacology; and the knowledge of disease and of its treatment has thus been put upon a sounder basis.

All this has followed the adoption of physics and chemistry as the guides of the physiologists.

In the present volume, Dr. Burns attempts to show the part which physics has come to play in the solution of the problems of physiology. A science of biophysics has evolved in the same way as that of biochemistry. Perhaps some attempt should have been made in the title to indicate that it is the problems of the physiology of vertebrates rather than the basic problems of life generally which are dealt with.

The book is intended for students of human physiology, although it cannot fail to interest all workers in biology.

It demonstrates what a very large number of the characteristic reactions of living matter may be explained in terms of ordinary physical processes, and it thus shows the reduction which is taking place in the number of phenomena which some are still content to explain as due to a mysterious vital action instead of simply confessing that they are yet not understood.

As the application of physics and chemistry to physiology is extended, it is safe to predict that fewer and fewer of these vital manifestations will remain unexplained.

The origin of living matter, its increase and dispersion all over the globe, its marvellous and endless developments and evolutions, and its reactions with its surroundings may all be explained in terms of physics and chemistry. But consciousness and its association with living things will ever remain the mystery it has been and is.

BIOPHYSICS

PART I. SYSTEMATIC

SECTION I.: ENERGETICS

CHAPTER I

LAWS OF ENERGY

"There is in reality only one general physics, only one chemistry, and only one mechanics, in which all the phenomenal manifestations of nature are included, both those of living bodies and those of inanimate ones." CLAUDE BERNARD.

"The history of man is dominated by, and reflects the amount of available energy." SODDY.

BIOPHYSICS deals with the application of physical and physico-chemical laws to the actions of living things. It is necessary at the outset to have a clear understanding of what is meant by a natural law, or principle of nature. A law in science is a different concept from a law in philology or in jurisprudence. Repeated observation of a recurring phenomenon leads to the conclusion that there is a natural and unalterable sequence of events. This is summarised in a law. Newton's Law, for instance, epitomises the conclusions of a large series of observations, viz. that objects free to do so always fall towards the earth. *A natural law, then, is not a principle governing the action of nature, but a generalisation drawn from observation of the phenomena,* stating, in short, how these phenomena have always been known to act in the circumstances. If the observations are correct, the law is true and, in like circumstances, will always hold. If at any time a reliable observation were made that seemingly went against the law, scientists would not doubt the validity of the law, but would carefully examine the concomitant circumstances to see in what point they differed from those defined in the law. The problem before us is to determine whether laws deduced from the study of non-living matter may be applied to the elucidation of biological phenomena. Physical science is the most fundamental of the experimental sciences and, so far as is known, its laws are applicable to all non-living matter. Biochemists have attempted to break

down the wall of partition which has been reared by common consent between the chemical constitution of living and non-living. They have been partially successful in that they have been able to build up certain typical products of life from non-living material. *No one, however, has, as yet, either analysed or synthesised living matter.* The finest chemical technique available cannot be employed without injury to the tissue studied. In spite of this drawback, the science of nutrition may be classed as exact. Mathematical formulæ may be employed to express results, and chemical response to a definite stimulus may be predicted.

Life has been compared to a flame. The Ancients looked on fire as a living thing, and is their view not, to some extent, justifiable ? The continual ringing of the changes—of form, of colour, or of position—by the flickering flames of our house fires draws the eye. Constantly, alterations are going on. No flame is still for any length of time. All is seemingly unordered and uncontrolled change. Yet down to the most minute movement all is governed by physico-chemical laws. Every flicker can be accounted for, and could be recorded as due to pressure of liberated gas, pressure and direction of draught, temperature of fire, etc. Fire —mysterious and all-powerful gift of the gods—has yielded to the prying endeavours of the scientist, and can be harnessed and employed in the service of man.

Similarly, while not committing oneself to a vitalistic or to a mechanistic conception of life, one may study, with considerable profit, the various physical phenomena exhibited by living matter. Examination of even the simplest form of life is sufficient to show that a characteristic phenomenon is change. No living thing is absolutely still. It is undergoing change in one way or another. It may alter its position relatively to its environment ; it may alter in its parts ; it may grow ; it may undergo alterations in internal (atomic or molecular) structure. The physico-chemical processes indicated by these changes or initiated by them are studied under the term metabolism (Gr. *metabole*, to change). Metabolism may consist in a building up of matter, anabolism (Gr. *ana* = up) or in the reverse process, a breaking down of matter, catabolism (Gr. *kata* = down). If anabolism is greater than catabolism, the organism grows ; if the two processes balance, the organism exists. The predominance of catabolism leads to disintegration. Complete immobility denotes death. Change indicates the utilisation of energy, which obviously must have come from some source outside the organism. " The mechanistic notion of life, the representation of the body as primarily and fundamentally a machine, is often bitterly and not very intelli-

gently opposed. We are told that the machine—the scientists' imitation of life—is not merely a purely inanimate mechanism. In its cunning combination of valves and regulators it has a brain, part of the brain of its designer. The partial likeness is that of the machine to the man, of the limited imitation to the original, not the other way about, which is true enough. But let us bear in mind one essential and undeniable fact. Machine or man, inanimate mechanism with the mechanical imitation of a brain, or brain controlling an animate mechanism, what of the power? The power to live, the power to do work is not in the brain nor in the body, not in the valves nor the moving parts. The power, whether of life or of mechanism, is *external*. This is the real ground of analogy" (Soddy). We must determine the source of this energy, study its laws, see how it is made available for living matter, and then see how it operates in living matter.

Energy is the underlying cause of all changes in matter. This does not seem a very satisfactory definition, but, so far, it is the only one possible. It is a very striking fact that the two fundamentals of our external world, matter and energy, have for us no existence apart from their effect on us. We cannot prove that there are such things except in so far as they manifest themselves, matter by being changed, and energy by producing changes, which in turn alter our sensation-complex. Energy, then, is that which produces an effect on our senses. Our sense organs, as we shall see later (Chap. XIX.), are stimulated by *changes* of energy in their environment. We see, because radiant energy of a certain frequency falls on our retinæ; we hear when the hair cells on the basilar membrane are stimulated as the result of air waves of definite frequencies transmitted to them *viâ* tympanic membrane or *viâ* bone; our senses of taste and smell depend on changes of chemical energy, while changes in kinetic energy produce the sensations of heat, cold, tickle, touch, pressure, position, etc.

Energy is measured by its power to do work. The kind of unit used will therefore depend on the nature of the work done. In the C.G.S. system the unit of work is the *erg*—that amount of work which is done when unit force is overcome through unit distance. The unit of force is the *dyne*, and is defined as the force which acting on a gram for a second would give it the velocity of one centimetre per second. An *erg* is, therefore, a dyne-centimetre. If the power developed is electrical it would be measured in *watts*, if mechanical, in *foot-pounds* per second, or in *horse power*, and so on. These various units may be converted into one another (Table I.). For our purpose it is most convenient to measure energy in heat units or calories.

In 1798, Count Rumford, who was engaged in boring cannon, showed that the energy of a moving body, *i.e.* kinetic energy, could be transformed into heat. Later, Joule demonstrated the equivalence of these two forms of energy. 427 kilogrammetres of work always produce (under standard conditions) one Calorie of heat. Conversely, heat may be transformed into mechanical movement. Indeed, any form of energy may be converted into any other form of energy. (Radio-active matter evolves energy which manifests itself in various forms, yet all attempts to change other forms of energy into radio-active energy or even to influence the rate of transformation have failed. Chap. XIII.)

TABLE I

EQUIVALENT UNITS OF WORK

1 Calorie or 1,000 gram. calories or 10^9 microcalories	equivalent to	$41 \cdot 9 \times 10^9$ ergs.
		$98 \cdot 8 \times 10^3$ foot-poundals.
		$42 \cdot 6 \times 10^6$ gram. cms.
		$3 \cdot 089 \times 10^3$ foot-lbs.
		4190 joules.

EQUIVALENT UNITS OF POWER

1 Calorie per second $= 41 \cdot 9 \times 10^9$ ergs per second.
$= 4 \cdot 19 \times 10^3$ watts.
$= 4 \cdot 19$ kilowatts.
$= 5 \cdot 61$ horse-power.

From observation it is found :

(1) That one form of energy may be transformed into any other form.

(2) That when any quantity of energy in any one form disappears, an exactly equal quantity of another form of energy makes its appearance.

Energy like matter is therefore indestructible (First Law of Thermodynamics).

Every substance possesses a certain amount of energy. This is called its internal or intrinsic energy. Further, every group of substances has associated with it a certain definite amount of energy as long as it remains unchanged. When any change takes place in the group, or in any member of the group, there is usually a corresponding change in its total energy, either an increase, due to the reception of energy from its environment, or a decrease, due to an evolution of energy. Put into other words, each of the new substances will have its own characteristic intrinsic energy, and the new group will, in general, have a different total energy-content from that of the original group.

E.g. Cane sugar $+ O_2 \rightleftharpoons CO_2 + H_2O +$ heat energy.

Corollaries of the First Law.

The following two deductions are of biological interest :

1. The total energy of a system *in a given state* is for the system in question a definite characteristic *of that state*, and it is totally independent of how the system reached that state.

The energy that would be liberated by the fall of a kilogram, *e.g.*, of lead, from a height of 300 metres would be the same no matter how the kilogram was first elevated to the point from which it was dropped. For instance :

(*a*) It might be lifted bodily and vertically.
(*b*) It might be lifted bodily and up an inclined plane.
(*c*) It might be lifted bodily and rapidly.
(*d*) It might be lifted bodily and with infinite slowness.
(*e*) Or it might be lifted in small pieces, say 1 milligram, at a time.
(*f*) *It might be lifted as a series of chemical compounds weighing more than* 1 *kilogram as salts of lead and reduced to metallic lead before dropping.*
(*g*) Or it might be dug out from the top of a hill, 300 metres high, and transported horizontally by aeroplane.

The essential conditions are that it weighs 1 kilo. and that it falls 300 metres, cosmic influences being constant.

Similarly, glucose has the same energy-content, no matter how it has been prepared, provided the measurement is carried out under similar conditions, *e.g.* glucose may be synthesised from simple substances ; it may be prepared by the natural or artificial hydrolysis of more complex carbohydrates, or it may be derived from such substances as proteins, fats, etc.

2. When a system changes from one state to another, the alteration of total energy which accompanies that change is altogether independent of the process by which the change is brought about.

Law of Hess.—This second corollary was enunciated by Hess in 1840 as the result of experiments. He worded it, " The amount of heat generated by a chemical reaction is the same whether it takes place all at once or in steps."

The examples given above, if reversed, will act as examples of this corollary. The rate and angle of fall are not determining factors in the liberation of energy, nor does the way in which the energy of glucose is liberated have any effect on the total amount set free.

These two corollaries, as we shall see later, make it possible to construct a balance sheet of the energy intake and output of the organism.

Degraded Energy (Second Law of Thermodynamics).

When a substance or group of substances is changed into a substance or group of substances with a smaller energy-content,

the energy thus liberated is, in theory, available for work. In practice, it is found that all this surplus energy cannot be recovered as work. No system is absolutely isolated and, though the total cosmical energy may be constant, its distribution and its state may alter. Some of the freed energy is *always* converted into heat, part of which is diffused among surrounding objects and is thus lost, as far as work is concerned. The *quantity* of energy is not decreased, but its dispersion is so great that in *quality* it has not sufficient potential gradient to be of use. As an example of dispersion of energy, consider how waves in ether, *e.g.* light or the longer (wireless) waves, fall away in strength in widening concentric zones as their influence spreads from the source, till at infinite distance the most sensitive apparatus can only with difficulty detect the disturbance. The wave energy has been so spread that it may be disregarded.

The second law is usually worded, " The entropy of an isolated system tends to a maximum." Entropy is a function which, while theoretically of great value as indicating the direction in which chemical or other processes take place, cannot be directly measured. Further, one never has, in Biology, to deal with an isolated system. The difficulty as well as the great interest of our science depends on the close interrelation and co-ordination of all the systems in it. A simple expression of the law, and one suited to our purpose, would be, " Every change takes place at the cost of a certain amount of available energy." The amount of energy so " degraded " during a transformation from one form to another may be taken as an inverse index of the efficiency of the transforming mechanism.

States of Energy.

A substance may be endowed with kinetic energy, or with potential energy, or with both. Kinetic energy is directly available for work, potential energy requires the use of some kinetic energy to liberate it. The energy of a substance may be in the motion of the substance itself or in the motion of the ultimate particles composing it. This kinetic energy and its value depend on the mass of the substance and the rate at which it moves or at which its particles vibrate.

Potential energy, on the other hand, is said to be possible to a substance in virtue of its configuration, *i.e.* position, composition, history, etc. A quantity of energy that may be measured is stored up (or rendered passive in some way), and this same quantity is theoretically recoverable in a measurable form. It may not be apparent *how* energy is stored up, but it may be demon-

strated that it *is* stored and *is* recoverable. The simplest example is the application of force to a perfectly elastic system. On removal of the force the system will return of itself to its original configuration, and an amount of work will be done by it, in returning, exactly equivalent to the amount of the force of distortion. The hackneyed example of energy storage is, of course, that of our coal supply.

There can be no liberation of energy without free energy. No change can take place without free energy. Our senses make us aware of free energy, but potential energy can be perceived only as the result of reasoning from past experiences. Potential energy, then, is a psychological concept.

Energy in the potential state, as long as it remains potential, is useless. It cannot be transformed into any other form of energy without altering its state, and its state cannot be altered without the employment of kinetic energy. This point is biologically important.

1. A weight hanging from a string has potential energy according to its mass and its distance from the centre of the earth, etc.

2. An explosive has potential energy depending on its chemical composition and physical state.

3. Petrol, coal, or any other fuel has similar energy bound in it.

4. A sleeping man may be said to have potential energy. In order to get work out of these quiescent bodies, all that is necessary is the application of a suitable and sufficient stimulus, *i.e.* a small quantity of free kinetic energy (pp. 222 and 235). *E.g.*

(1) The resistance that prevents the weight from falling must be overcome, *i.e.* the string severed.

(2) The explosive must be fired or detonated.

(3) The fuel must reach ignition temperature.

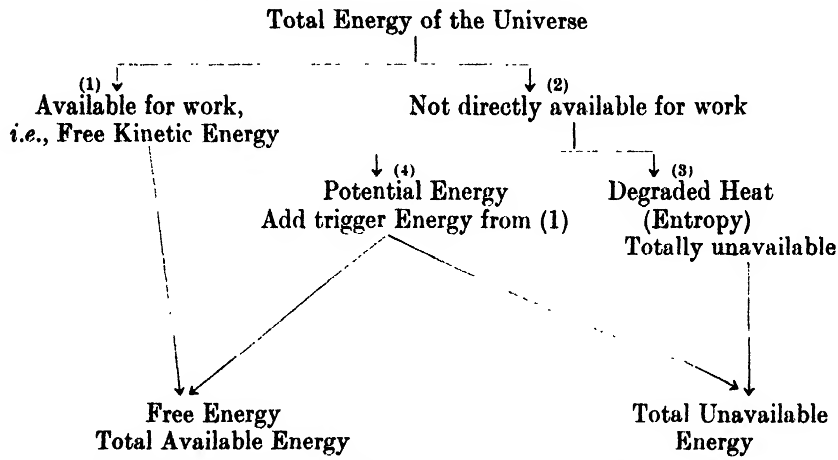
(4) The sleeper must be awaked.

Free and Bound Energy.—In 1882 Helmholtz introduced the terms " free " and " bound " to denote respectively that part of the kinetic energy of a system free or available for conversion to work, and that part not free or not available for this purpose. Later writers, realising that potential energy as such was not available for work, widened the connotation of " bound " to include this dormant energy. As a matter of logical definition, only potential energy is really bound. That which is bound can be freed by cutting the bonds, *i.e.* by doing an amount of work which bears no relation to the amount of energy bound, but depends on the nature of the bonds. Dissipated or degraded energy is not in any true sense bound. It is not free to do work, *i.e.* it is not available. To render it available an amount of energy would have

to be expended on its environment at least equal to the amount of energy rendered available.

A weight resting on the ground may be considered as representing a body having degraded energy. Its energy potential is the same as its environment. No work could be got from it without the previous expenditure of work on it. If a certain amount of work were done in raising it from the ground, then the same amount of work would be recovered on letting it fall to the ground (taking into account the mechanical equivalent of the degraded heat). On the other hand, a weight resting on a ledge above the ground may perform work in falling if sufficient free energy be applied to tip it over. The quantity of work done in tipping over the weight bears no relation whatever to the amount of energy liberated in falling.

The following scheme may help to make the matter clear:



(3) Called "bound" energy by Helmholtz in 1882.
(3 + 4) Called "bound" energy by later workers.
(4) Called "bound" energy by Physicists.

One of the most important problems in biology is the means by which potential energy is translated into work and the mechanism by which this translation is controlled.

"**The struggle for existence is the struggle for free energy**" (Boltzmann).

Of potential energy there is an abundant supply. Some of it, *e.g.* that of coal, requires the employment of only small quantities of free energy to render it immediately available for work, while other varieties, *e.g.* that of radio-active minerals, have their energy bound in such a way that it is evolved with excessive slowness. Uranium contains the same amount of energy as 250,000 times or more of its weight of coal, but little more than $\frac{1}{10,000,000,000}$ part

of this is given out in a year (Chap. XIII.). To enable mankind to avail himself of this kind of energy, some means will have to be devised for speeding up and controlling the output. As Professor Soddy puts it: "Primitive man froze on the site of what are now coal mines, and starved within sound of the waterfalls that are now working to provide our food. The energy was there, the knowledge to utilise it was not. So while we are leading cramped lives and fighting among ourselves, whether in peace or war, for a modicum of the means of existence, science tells us that, in the commonest materials that make up the framework of the world, there is energy of a magnitude of which we have no experience, and the means of livelihood of which we have no standard. The energy is there. The knowledge that can utilise it is not—not yet."

Physiological availability.

This introduces a further point. The energy-content of cellulose is much the same as that of starch, yet as a source of energy for man the former substance is useless, while the latter is perhaps his main source of energy supply. An inorganic example may make this clearer. Two lakes may be exactly similar except that one has an outlet, while the other is surrounded by impassable mountains. The water power, *i.e.* the stored energy of the former, is utilisable, while the latter could not be tapped without arduous engineering labours. The energy-contents of the radio-elements (atomic energy) of cellulose (as human food) and of the undrained lake are said to be non-available. Future scientists may discover how to draw upon this surplus energy supply.

Principle of Le Chatelier.

"*Every system in chemical equilibrium, under the influence of a change of any single one of the factors of equilibrium, undergoes a transformation in such direction that, if this transformation took place alone, it would produce a change in the opposite direction of the factor in question. The factors of equilibrium are temperature, pressure and electromotive force, corresponding to three forms of energy—heat, electricity and mechanical energy.*"

The above is the principle as enunciated by Le Chatelier in 1884. Consider the energy changes in the formation of water where hydrogen and oxygen combine with the evolution of 68·8 Calories for every gram molecule formed. The equation of the reaction may be written

$$2H_2 + O_2 \rightarrow 2H_2O + 136{\cdot}6 \text{ Calories.}$$

But at high temperatures the reaction is reversible, that is, H and O are formed from the water :

$$2H_2O + 136{\cdot}6 \text{ Calories} \rightarrow 2H_2 + O_2$$

Both reactions take place simultaneously, some of the water being decomposed steadily and some water being formed, so that an equilibrium is established between these two opposing forces :

$$2H_2 + O_2 \rightleftharpoons 2H_2O + 136{\cdot}6 \text{ Calories.}$$

If we add either H alone or O alone to the system, the equilibrium is shifted in the direction of the *upper* arrow *so that some of the added constituent undergoes combination.* Similarly, if we alter another of the intensity factors, say, temperature, and apply heat to the reacting substances, the equilibrium is shifted in the direction of the *lower* arrow and *heat is absorbed.* The principle of Le Chatelier enables a prediction to be made of the direction in which a reaction to a disturbance may take place. Only those predictions are valid which involve changes in the intensity factors of the energy of a system.

The principle has been broadened in order to apply it to the *dynamic* equilibrium of living things. For example, almost simultaneously with the publication of Le Chatelier's paper, Frédéricq stated, " The living organism is such that each disturbing influence produces in the organism itself a compensatory reaction which tends to neutralise the disturbance and restore the equilibrium." Now Le Chatelier postulated that the systems to which his principle applied were to be in *stable* equilibrium, and so one cannot justly apply his name to Frédéricq's interesting hypothesis.

Inertia.

The second law of energetics lends itself to the deduction that the cause of all action (change) is the tendency of energy to attain the same uniform degree of intensity as that of its environment. Further, the degradation of energy follows the line of least resistance. This is known as the " Law of Least Action." It is a law common to all sciences, and is considered by some to be a universal principle. Physicists tell us that bodies remain in a state of rest or of uniform motion in a straight line unless energy be imparted to them to overcome their inertia. Inertia is the resistance to change possessed by everything, living and non-living.

One may take a step further and qualify the law by stating that the nature of the change induced by an alteration in any factor which influences the system will depend on what will, in the circumstances, give relief from strain with the least possible expenditure of energy. This law was stated by Berthelot in a

somewhat different form, and is known as the principle of the necessity of reactions. It is, "Every chemical change which is accomplished without the addition of external energy *necessarily occurs if it is accompanied by a disengagement of heat.*" This means that, where possible, substances which can react spontaneously do so, if the products of the reaction contain less energy than the reacting substances. In other words, water, if allowed, runs downhill. These two principles are important generalisations for the biologist, but there are many exceptions to them. When a state of strain is made more or less permanent, the organism readjusts itself to meet the strain. That is, the easiest course is not to remove the cause of strain, but to make such an alteration in itself as shall render the external change innocuous. This is the principle underlying the theory of adaptability. A tree arranges its branches so as to offer least resistance to the prevailing wind. Other examples might be drawn from the sciences of physiology, economics, psychology and ethics (Chap. XVII.).

Physiology. The introduction of an irritating substance into the alimentary canal causes vomiting to remove the cause of irritation, *i.e.*, to relieve strain. Some less exhausting means of relieving strain has to be taken to meet the more or less continuous administration of poison. The cells of the organism so alter as to be immune from such irritation. Mithridates is said to have qualified for the throne of Pontus by the ingestion of all sorts of poisons in his youth.

Economics. The law of supply and demand, rates of exchange, etc., are merely restatements of this principle of least action.

Psychology and Ethics. The unjust judge met the early appeals of the widow with a firm refusal. His mind was relieved, his case settled. Because of her very importunity, persistent strain was set up which had to be relieved by reopening the case and giving a just decision.

Enough has been said to show the possibilities of this deduction from the second law of energetics. The thorough-going mechanist states that this law of least action is the principle governing the action of living as well as dead matter.

All action, it is said, is a response to stimulus, and is such as will most permanently and with "least action" relieve the state of strain. The mechanist denies any cause of action but this. What has been taken for the effect of will or instinct is in reality the effect of light, of gravity, of friction, of chemical force, or of some other known or knowable external force. In short, some alteration in an external factor has brought about an instability in the physico-chemical equilibrium of the object or of the organism, and thus a shift in the equilibrium will take place in such a direction as to decrease the magnitude of the alteration which would otherwise occur. The animal, human or otherwise,

is but a machine, working according to physico-chemical principles, reacting blindly and quantitatively to every chance force which plays on it. While, to a certain extent, we may regard this as true, we must, nevertheless, draw a sharp distinction between those actions which may be regarded as pertaining to organic life and those which pertain to conscious life dominated by personality. Plants and animals may be governed by this law of equilibrium, and every one of their actions may be regarded as a blind response to stimuli, just as the swing of a galvanometer needle is. Man, in so far as he is an animal, may also be considered a blind agent. Is there not, however, something superadded—not to interfere or even to govern, but to carry out a function of its own ? For example, there are no grounds for dealing with volition merely as a complex chemical equation or as a problem in molecular physics, resulting merely from physical or chemical changes in the body or environment. Suppose a man meets another man in the street who suddenly strikes him. The injured man has several courses open to him :

1. He may hit back.
2. He may run away.
3. He may fetch a policeman, and so on.

The action taken depends on several factors :

1. The previous history of the two men.
2. The relative sizes of the two men.
3. The nature of the spectators.
4. The nearness of the policeman.
5. The real business of the injured man, whether pressing, etc.
6. The personality of the injured man.

Knowing the man one may predict his action in a certain case, and one may probably be right, but it is only a probability—not a certainty. While the cause of volition is still unknown and cannot but be regarded as mysterious, there is nothing to hinder research into the mechanism whereby the Will causes its *dicta* to become *acta*.

To summarise, the physical necessities of man have become a problem of energy pure and simple. The fact that man is living scarcely makes the problem more complicated than one arising out of the fuel demands of an inorganic machine. So much work has to be done, so much energy must be provided.

Energy is indestructible, and in itself is only valuable in its conversion from what may be called higher to lower forms. This transformation involves the loss of some available energy. A

certain amount finds its way into the great ocean (or " sink ") of heat energy of nearly uniform temperature. The attainment of this dead level is the final goal of all energy whether it is utilised or not.

Further Reading

Soddy. " Matter and Energy." Williams and Norgate.
Lotka. " Elements of Physical Biology." Williams and Wilkins.

CHAPTER II

THE STORAGE OF ENERGY

"All forces of the earth, all manifestations of life are modulations and variations of the same heavenly melody which proceeds from the sun." TYNDALL.

ALL life processes demand for their continuation and maintenance a continuous supply of matter and energy. As far as matter is concerned, there is a closed cycle. Animals feed on plants, and plants feed on the products of animal metabolism and disintegration. Energy, however, must be supplied from *outside* the cycle. The one essential physical factor that makes the process possible is the supply of energy as sunlight to the plant.

The ultimate source of all the energy upon which existence on this planet depends is the sun. (One need not here enter on the

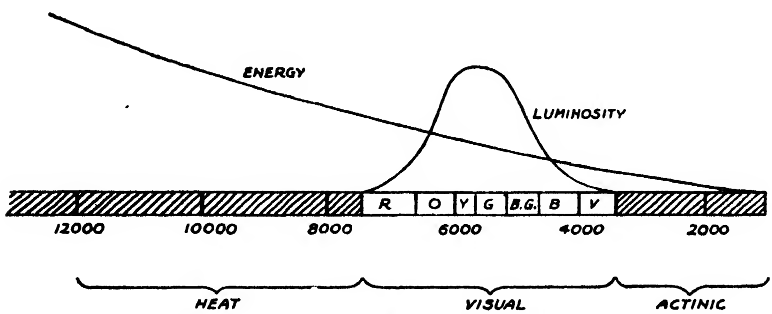


FIG. 1.—Curves showing relative energy and luminosity of different regions of the spectrum. (Abney.) The wave-lengths are given in Ångström units. An Ångström unit (A.U.) = one ten millionth of a millimetre = 0·1 $\mu\mu$

interesting question of how the sun evolves energy; see Soddy, *Matter and Energy*, Chap. X.) As far as we know, the higher forms of life are unable *directly* to use either heat or light as sources of bodily energy. Some of the lower forms of animal life may have this power; plants certainly have. As we shall see later, light may act as trigger-energy starting a series of changes in matter and its energy content whereby a liberation of free energy is effected. This free energy will then be available for work.

The green plant is able to collect and conserve a portion of the stream of energy emitted from the sun. This it does by virtue of its content of a green pigment chlorophyll dissolved in the lipoids

of the plastid. The existence of chlorophyll in fossil vegetation shows that it is of primary importance in the evolution and maintenance of life. Man, lord of the earth, depends for his very existence on the presence of an organic magnesium compound in the humble grass of the field.

The light that falls on the leaf may be (*a*) reflected, (*b*) refracted, or (*c*) absorbed. It is obvious that only absorbed energy can have any effect on the metabolism of the leaf. This statement is known as Grotthus' Law, and can quite readily be proved. For example, a substance appears red to us because it is *reflecting* light whose mean frequency is 375×10^{12} cycles per second. It will, therefore, absorb light of other frequencies. Grotthus showed that *red* iron thiocyanate underwent no change on exposure to *red* rays, but was bleached when green light fell on it. Similarly the blue complex formed by the action of iodine on soluble starch is decolourised by yellow light and yellow gold choride by blue light.

Absorption Spectrum of the Green Leaf.

A mere glance at the absorption spectrum of the green leaf is sufficient to show that the light best absorbed is that having a wave-length less than 500 $\mu\mu$, the amount absorbed becoming greater as the wave-length becomes shorter, *i.e.* the chloroplasts absorb the actinic rays (violet and a small amount of the ultra-violet rays). There is also a well-marked absorption band in the red portion of the spectrum between 665 and 685 $\mu\mu$. The figure for the maximal energy of solar radiation is given by S. P. Langley as 650 to 666 $\mu\mu$ for high sun, so that the green leaf is able to (*a*) utilise the actinic rays, and (*b*) absorb light of that wave-length (red) which is emitted by the sun in greatest amount. The pigments of the chloroplast do not utilise green-yellow light, nor do they absorb the heat (infra-red) rays at all.

Consider next the physical (and chemical) changes brought about by the absorption of light. Although this is the primary problem in Biology and has attracted many investigators, it remains unsolved. Research has made it more apparent that the mechanism for converting solar radiation into bound energy is not so simple as was at first thought. Certain facts, however, have been brought to light.

(1) Matter is assimilated. Elements taken from the environment are built into organic compounds. Boysen-Jensen has shown that in July the accumulation of matter (dried) may reach 16·5 per cent. of the total dry weight of the plant. A large proportion of this matter can be shown to be **carbohydrate** by a very simple experiment. It is only necessary to screen a portion

of a leaf from the light, leaving a certain portion exposed, and then observe any differences between the normal and the darkened portions. If a leaf, like sunflower or fuchsia, is chosen and previously kept overnight in the dark, exposure to light for 15 minutes is sufficient, and one hour is more than ample for our purpose. The leaf is then bleached with warm alcohol, and iodine is applied. The part exposed to light will appear blue-black—rich in starch, while the screened portion is starch free. It has been shown that during the day the starch content of leaves may rise to 6·44 per cent. of the dry leaf weight. At night the starch value may drop as low as 0·38 per cent. Timiriazeff has devised a very neat experiment which demonstrates that starch formation is greatest where there is greatest absorption of light. Living hydrangea leaves, previously deprived of starch by retention in the dark, have then projected on them a solar spectrum for 5 or 6 hours. The leaf is decolorised and treated with iodine, and then the absorption bands of the chloroplast pigment complex are found mapped out in blue, showing that starch has been formed only where light has been absorbed. Other carbohydrates are also found. Cane sugar is formed and can be detected before starch can be found, and it is generally present in greater amounts, 7·63 per cent. to 2·63 per cent. of the dried-leaf weight. Other sugars are present in small variable quantities.

(2) It seems that CO_2 is absorbed beyond the needs of respiration, and that O_2 is evolved. Engelmann has provided in a striking manner a demonstration of the fact that the maximum evolution of oxygen takes place where there is the maximum absorption of light and, as stated above, the maximum formation of starch. He placed a filament of cladophora in water, to which he added some motile bacteria having an avidity for oxygen. On the thread of alga he projected a minute solar spectrum and kept it under the microscope. It was seen that the bacteria gathered just at those places (red and violet) where light was absorbed.

Kniep and Minder have determined the carbon assimilation, and they find it directly proportional to the amount of energy absorbed as light. Further, Willstätter and Stoll have estimated

(*a*) The CO_2 taken up by a leaf area in the dark (*respiratory* CO_2).

(*b*) The CO_2 absorbed in light of a definite intensity (*total* CO_2).

Total CO_2 − respiratory CO_2, *i.e.* $(b - a)$ = *assimilated* CO_2.

(*c*) The O_2 evolved in the dark (*respiratory* O_2).

(*d*) The O_2 evolved in the light (*total* O_2).

Total O_2 − respiratory O_2, *i.e.* $(d - c)$ = non-respiratory O_2.

(e) $\dfrac{(b-a)}{(d-c)} = \dfrac{CO_2}{O_2}$ = assimilation coefficient.

(Cf. respiratory quotient, see Chap. III.).

They find that under all conditions of light the assimilation coefficient is 1. That is, for every volume of CO_2 assimilated, there is evolved a volume of O_2. In short, the chloroplast acts as a machine for converting CO_2 into C and O_2 which is evolved. The carbon then unites with water to form some simple sugar:—which one is not known. The final product is starch. The process may be represented by the equation:

$$xH_2O + xCO_2 + \text{light energy} = C_xH_{2x}O_x + xO_2.$$

It has been proved that the first step is the formation of formaldehyde, *i.e.* $x = 1$.

(i) $H_2O + CO_2 + 112{,}090$ gram cals (?) $\rightleftharpoons O_2 + CH_2O$ (formaldehyde).

Formaldehyde is injurious to plant tissues, and it is rapidly transformed into other products.

If $x = 2$ then the formaldehyde would condense to form glycollic aldehyde—a diose.

(ii) $CH_2O + CH_2O + \text{energy} \rightleftharpoons C_2H_4O_2$.

Similarly, glucose could be formed.

(iii) $C_2H_4O_2 + C_2H_4O_2 + C_2H_4O_2 + \text{energy} \rightleftharpoons C_6H_{12}O_6$.

Two molecules of glucose combine to form maltose.

(iv) $C_6H_{12}O_6 + C_6H_{12}O_6 + 3{,}300$ gram-calories $\rightleftharpoons C_{12}H_{22}O_{11} + H_2O$.

(v) The gums and dextrins are composed of condensed molecules of maltose, *e.g.*

$$C_{12}H_{22}O_{11} + C_{12}H_{22}O_{11} + H_2O + \text{energy} \rightleftharpoons C_{24}H_{46}O_{23},$$

and so on.

(3) Carbon-dioxide and water have small energy contents (2·1 and 6·5 gram calories per gram respectively, and these amounts are not available for food), while starch has a value of 4·1 large Calories per gram. It is evident, therefore, that in some way the plant has converted a certain amount of kinetic energy into potential energy. Now, as the formula for starch is uncertain, let us consider the amount of energy required to form glucose from CO_2 and H_2O. Carbon dioxide and water are fully oxidised. Theoretically, they may be considered as undergoing a process of reduction before combining to form the aldehyde, but as the energy evolved during reduction would be balanced by the energy absorbed

during formation, we may limit our problem to the total energy change according to the two equations given above, viz. (i) and (iii). From (i) we see that a gram-molecule of formaldehyde has an energy-content approximating 112,300 gram calories. This store of energy is derived from the constituents H_2O (117 gram-cals.), CO_2 (92·4 gram-cals.), and from absorbed sunlight (112,090·6 gram cals.).

With the formation of formaldehyde, practically all the energy necessary for the formation of carbohydrates has been absorbed. As we shall learn later (Chap. V.), osmotic energy is a function of particular concentration. Therefore, when six molecules of formaldehyde are condensed to one molecule of glucose, a corresponding amount of osmotic energy is liberated, and this may be utilised in part in endowing the glucose with the slightly higher content of chemical energy which it possesses over that of the formaldehyde. Sunlight here acts as a catalyst (Chap. X.). Moore and Webster have synthesised formaldehyde by exposing an aqueous solution of CO_2 to ultra-violet light (Chap. XIII.) in the presence of inorganic colloids (Chap. VIII.).

As we have seen, all the light falling on the leaf is not utilised —even all the light absorbed is not stored. Some energy is required for direct domestic use, *e.g.* transpiration. It has been calculated that about 10 per cent. of the incident light is absorbed by the chloroplast pigments. In an experiment by Brown and Escombe it was found that a total amount of incident light, which, if converted into heat units, would correspond to 0·041 cal. per sq.cm. per minute, caused the decomposition of 0·00034 c.cm. of CO_2 per sq.cm. per minute. In the conversion of 1 c.cm. of CO_2 into glucose 5·02 gram cals. are stored. Therefore, in building 0·00034 c.cm. of CO_2 into sugar the amount of energy rendered potential would be $0{\cdot}00034 \times 5{\cdot}02 = 0{\cdot}0017$ gram-cals.

Total incident light per sq.cm. per minute = 0·041 cals.

Total volume of CO_2 per sq.cm. per minute = 0·00034 c.cm.

Energy rendered potential per sq.cm. per minute = 0·0017 cals.

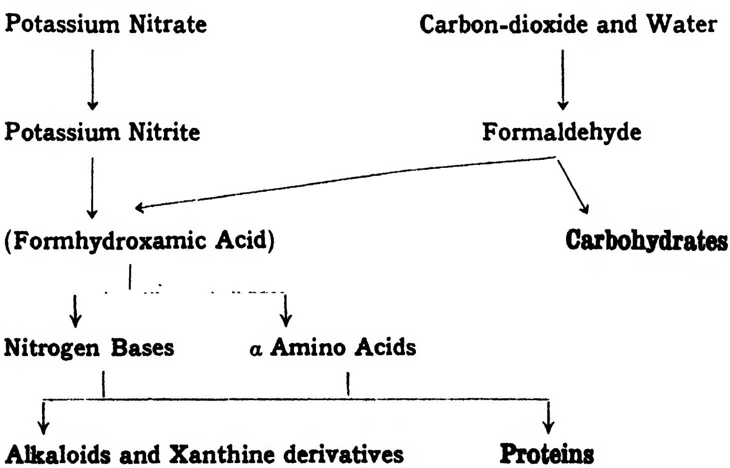
That is, 17 cals. out of every 410 that fall on the leaf are stored in starch. But as only 41 cals. (10 per cent. of the total incident light) are absorbed, we may consider that the efficiency of the chloroplast under maximal conditions is somewhere about 40 per cent. When the process is reversed and carbohydrate split up with the assimilation of oxygen and the evolution of carbon-dioxide this energy is again set free. It may be freed in such a way that a certain proportion of it appears as light. This light has, according to Trautz, the same wave-length as the originally absorbed light. Of course, in general, the energy will be evolved in a form more

suitable for utilisation than this (see Chaps. on Osmosis, Surface Tension, etc.).

Fats are stored up also in the plant. Very little research has been done on the synthesis of fats in the plants. Whether the plant can form these compounds directly or whether they are only synthesised from carbohydrate is not known. That they can be formed from carbohydrates is known, and Leathes states that this action is exothermic, several molecules of simple sugar going to the formation of one (larger) molecule of fat, having, of course, a higher caloric value. The fat is almost exclusively found in the fruit.

Incidentally, energy is bound in the formation of **proteins.** This energy comes indirectly from the sun. Atmospheric nitrogen is fixed in a form available for plant use by certain bacteria. Each gram of nitrogen so fixed carries with it a considerable quantity of energy which is obtained from the oxidative decomposition of 100 mg. mannitol, the parent alcohol of the carbohydrate, mannose. Moore and Webster have also shown that dilute solutions of nitrates exposed to ultra-violet rays are converted into nitrites with an absorption of energy. One gram molecule of nitrite formed from nitrate transforms about 10,000 gram-calories of radiant energy into the potential state—a strong endothermic reaction. This is similar to the change taking place in the plant in the formation of nitrogen compounds—the first stage in protein anabolism.

Baly suggests the following scheme as an indication of what probably occurs in the plant.



To conclude, the plant acts as a transformer of kinetic into potential energy by the formation of carbohydrates, fats, proteins (the so-called

proximate principles of food) and a few other substances of minor importance as storehouses of energy.

Having regard to the fact that free energy is of vital importance, and that the potential energy of the foodstuffs is readily rendered available, one would consider it a profitable study to determine the exact mechanism of this conversion. So far, study of pure chlorophyll has led to negative results. Kremann and Schnidlerschitsch have shown that pure chlorophyll, in alcohol, absorbed the same amount of CO_2 as the alcohol itself, and it

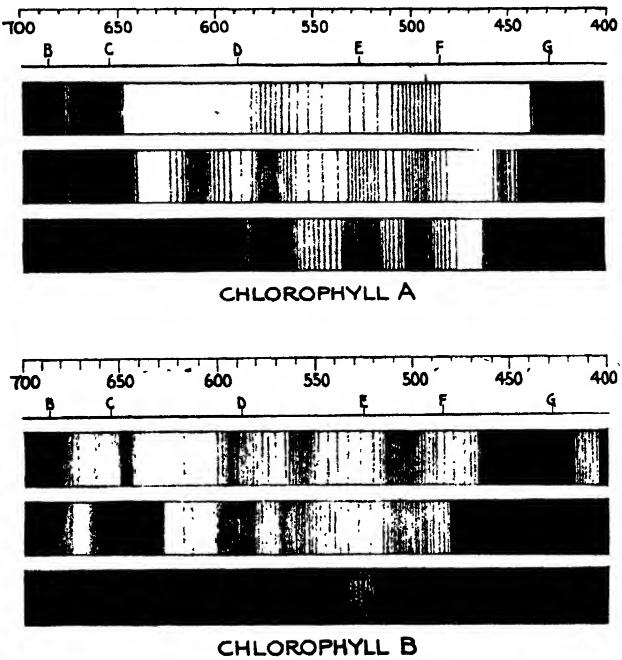


FIG. 2.—Absorption spectra of chlorophylls A and B. 0·0312 gram in ether Thicknesses 5, 20 and 40 mm. (After Willstätter, Stoll and Utzinger.)

made no difference whether the solution were exposed to light or kept in the dark. The absorption spectrum of neither chlorophyll *a* nor chlorophyll *b* nor chlorophyll *a* + *b* is quite similar to the spectrum of the living green leaf. Knowledge is incomplete both of the chemical nature of the various constituents of the chloroplast and of the distribution and physical state of the components of this heterogeneous system. The pigments are associated with a colloid complex: and the absorption of CO_2 is accompanied by alterations in the electrical state.

Moore and his co-workers have proved that inorganic colloidal uranium, iron and aluminium hydroxides act as catalysts in this

conversion of kinetic to potential energy. They also found that iron is present in the *colourless* part of the chloroplasts of the green plant. Sun and iron are necessary for the development of the chlorophyll although the pigment itself contains no iron. It, however, according to Willstäter, contains *magnesium* as an integral part of the molecule. Fenton, in 1907, showed that carbon-dioxide and water could combine to form formaldehyde when reduced by metallic magnesium. From Moore's work, it may be inferred :

(1) That inorganic colloidal systems evolved in point of time before organic colloids.

(2) That under the influence of sunlight *and in the presence of an inorganic catalyst,* the chlorophyll system developed.

(3) The inorganic system is able to utilise light only of short wave-length, while

(4) The chlorophyll system acts as a transformer (p. 164) and utilises the more abundant longer waves (Fig. 1).

To sum up, man obtains the energy necessary for his maintenance and for the performance of physical work from the disruption of proteins, carbohydrates and fats, synthesised in the first instance by green plants which trap and store solar energy. Historically, and until quite recently, the energy of sunlight, apart from an insignificant amount drawn from the tides, was the sole income of energy available for the world. Mankind still maintains himself solely on the energy derived from the sun through the intermediary of plant and animal metabolism, but he derives his energy for work to an increasing extent from a legacy of potential energy laid by in former times. He has devised detachable limbs (machines and tools) able to utilise the energy of coal, petrol, etc., of which he could not avail himself without their aid. This has made possible an enormous increase in the world's work—work done no longer by human beings and beasts of burden, but by inanimate machines using the energy of fire, electricity, etc. To-day, a single machine does the work of an army of men. In this way man conserves present-day solar energy and lives on the banked income of past ages. Some time in the future he may learn how to synthesise food from inorganic constituents by the use of any form of energy available. Then and only then will he be able to dispense with plant life. The energy available for each man is his income. Stored energy is a legacy deposited in Nature's " bank."

Further Reading

Moore. " Biochemistry." Edward Arnold.

CHAPTER III

LIBERATION OF ENERGY

(1) CALORIMETRY

"From the use of materials arise physical results, such as work, heat and electricity, which we can express in heat units. This is the power derived from metabolism." VOIT.

THE next matter for consideration is the method of measuring the potential energy of foodstuffs and comparing the value so found with the actual amount of energy liberated in the organism. It should then be apparent whether living matter in its various energy-changes obeys the laws of energetics. For purposes of measurement, it is customary in biology to convert all forms of energy into that of heat. This is scientifically correct, as heat is the "lowest grade" of energy, and all other forms of energy (ordered motion) may be degraded to unordered motion (heat); and it is not possible completely to convert any form of energy into any other form of energy but heat. The unit of heat adopted in biology is the large Calorie—that is, 1000 times the amount of heat required to raise one gram of pure water from 15° to 16° C. This value is almost the same as that required to raise one kilogram of water through 1° C.

Just as a country must have a standard coin of the realm—pound or dollar—in which its assets may be computed, so must there be a standard unit for the computation of energy. The bank-teller totals up his day's transactions in £ s. d. or $, no matter how various are the forms in which he has received or parted with the money. Cash, notes, cheques, deposit receipts, etc., all appear on his final balance-sheet under one denomination. Similarly, all energy transactions can be summed up and balanced as so many Calories received, so many Calories expended. Further, the fact that not a single sovereign may have crossed the counter does not hinder the banker from entering £ sterling in his books. So Calories may be the units employed, although heat may not necessarily enter the reaction.

A. Measurement of energy-value (E.V.) of foods by ultimate analysis.

The energy of a pure chemical compound may be calculated

from its chemical formula. The amount of heat evolved when C is oxidised to CO_2 and when 2H is combined with O to form water has been determined. The equations of these two reactions could therefore be written :

$$\underline{C} + \overline{O_2} = \overline{CO_2} + 94{\cdot}31 \text{ Cals.}$$
$$\overline{2H_2} + \overline{O_2} = 2H_2O + 136{\cdot}6 \text{ Cals.}$$

(A horizontal line above the formula of a substance in a thermo-chemical equation indicates that the substance is in the gaseous state, the absence of any line indicates the liquid state, while a line below the formula indicates the solid state. The suffix aq is intended to convey the idea that the substance is in solution in such a large volume of water that the addition of more water would not produce any appreciable effect—that is, the substance is so dilute that its heat of dilution on the further addition of water would be negligible.)

One must note that any alteration of gaseous volume or of any other physical characteristic of any of the reacting units would, by utilising some energy as positive or negative work, produce an alteration in the amount of heat evolved. Welter enunciated a rule whereby one might arrive at an approximate value of the heat of oxidation of a compound containing oxygen as well as carbon and hydrogen. According to this rule the oxygen is subtracted from the molecular formula with as much hydrogen as would serve to convert it completely into water; the heat of oxidation of the carbon and hydrogen in the residue then gives a rough value of the heat of oxidation in the whole compound. For example :

Tri-olein has the formula . . .	$C_{57}H_{104}O_6$
Deduct intramolecular water ($6H_2O$) .	$H_{12}O_6$
Leaving for oxidation	$C_{57}H_{92}$

Heat of combustion $= 57 \times 94{\cdot}3 + 92 \times 34{\cdot}15$
$= 5375{\cdot}1 + 3141{\cdot}8$
$= 8517$ Calories.

That is, a gram molecule (884 grams) of triolein in being completely oxidised to $CO_2 + H_2O$ would liberate 8517 Cals. of heat, approximately 9·3 Calories per gram. Similarly, the energy stored in the form of carbohydrate may be calculated :

Glucose	$= C_6H_{12}O_6$
Intramolecular water	$= H_{12}O_6$
For oxidation	$= 6C$

$6C + 6O_2 = 6CO_2 + 566$ Calories.
approximately 3·2 Calories per gram.

The value found by direct combustion is 3·7, a considerably higher figure. A difficulty, however, occurs with proteins.

In the case of carbohydrates and fats the end-products are

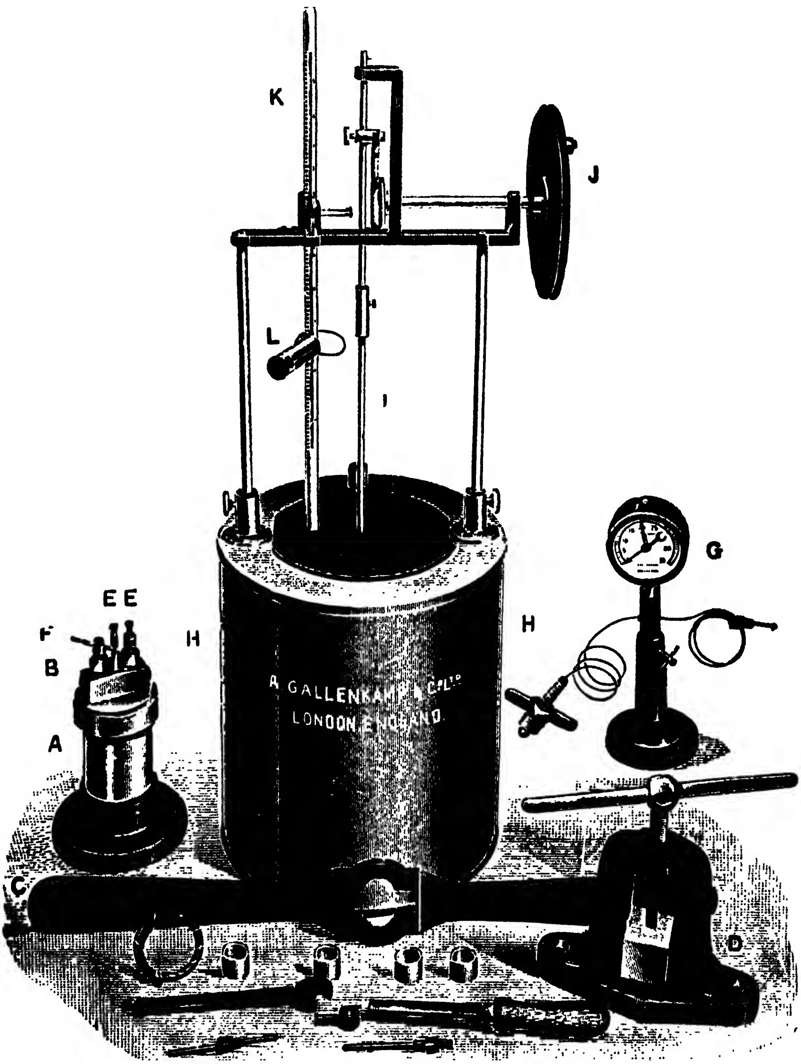


FIG. 3.—Berthelot-Mahler Bomb Calorimeter.
A. Bomb or autoclave (see Fig. 102).
H. Insulating vessel.
G. Manometer on stand with unions to fix to oxygen cylinder and to bomb (at *S*, Fig. 102).
D. A steel mould for making pellets from the material to be burned.

those of complete oxidation, but in the case of proteins, the final results of metabolism are not substances of the lowest energy content, *i.e.* protein is not completely oxidised. Further, these protein end-products are eliminated in solution, and this

requires some correction. Finally, to obtain the correct content of the foodstuff of C, H and O requires a complicated chemical technique, and the caloric value of an element may vary with its position in the molecule. For example, Barker states that

	the E.V. of the CO group is		60·7 Calories	
and	,, ,,	OH ,,	12·9	,,

but the EV of a COOH group may not be obtained by adding 60·7 and 12·9. This investigator finds that *carbon* has lower calorie values when it is arranged in such a position in an organic chain that its bonds approach the tetrahedral position. This method of calculating energy-value is little used in biology. Pure substances are seldom used as food material, except in certain kinds of experiments.

B. Measurement of E.V. of foods by calorimetric combustion.

The principle underlying this method is the combustion of a known amount of the material in an apparatus so devised that practically all the heat evolved is absorbed by a known amount of water and by the apparatus itself (which is of known heat capacity). Some form of bomb calorimeter is now universally employed for this purpose. The instrument (Fig. 3) is described on p. 24.

On p. 5 we mentioned the Law of Hess, which enables us to apply calorimetric combustion values to the food used by an animal. Provided the final products of combustion in the bomb are the same as the final products of metabolism, the energy liberated from equal quantities of the same food material will be the same. In the bomb the process of oxidation is rapid, in the body it is slow. In the former case the intermediate steps are known, in the latter they are not, but if the carbon becomes CO_2 and the hydrogen H_2O in each instance, the same quantity of energy will have been evolved. This law also permits us to calculate the energy set free when the process stops before oxidation is complete. For example, if C were only to combine with one instead of two molecules of oxygen and we found from actual combustion that:

$$(1)\ C + O_2 = 94{\cdot}3 \text{ Cals.}$$

and

$$(2)\ \tfrac{1}{2}(2CO + O_2) = 68{\cdot}1 \text{ Cals.}$$

Then $(1 - 2) = 26{\cdot}2$ Cals. would be the heat liberated in the reaction $\frac{1}{2}(2C + O_2) = CO$. This principle is of great importance in arriving at a caloric value for proteins (see p. 26).

Determinations of the energy-value of the proximate principles of food have shown that only minute differences exist between the various members of any class. For example, one gram of

each of the following carbohydrates has the accompanying value in calories :

TABLE II

Starch	= 4·191 large Calories.
Cane Sugar	= 3·955 ,, ,,
Maltose	= 3·94 ,, ,,
Milk Sugar (Lactose)	= 3·95 ,, ,,
Glucose	= 3·74 ,, ,,
Fruit Sugar (Fructose)	= 3·75 ,, ,,

Average values have therefore been adopted and accepted as standard. *E.g.*

Carbohydrate	4·1 Calories.
Fat	9·3 ,,
Protein (Physical value) . .	5·3 ,,
Protein (Physiological value) .	4·1 ,,

Of course, the discerning student will understand that, except in rare and restricted feeding experiments which have a special end in view, pure carbohydrate, fat and protein are not exhibited. Determinations are made of the energy-value of actual foods. This gives opportunity for the display of some ingenuity on the part of the investigator, since some of the commoner articles of diet do not readily lend themselves to combustion and are not easily dried. Nevertheless, extended experiments are being conducted by physiologists, in which, as part of the routine, the total energy-value of the daily diet is determined.

The energy-value of the diet does not necessarily represent the energy *used* by the organism.

(*a*) Some energy-carrying substances cannot be digested, and therefore are excreted unchanged in chemical composition and energy content, *e.g.* cellulose.

(*b*) Other constituents of the diet may undergo some chemical alteration, but may be excreted not fully deprived of their energy.

(i) Proteins are not completely oxidised in the body. Their end-products are urea and allied substances.

Because of the difference in the end-products there is a **physiological calorie value** for proteins different from their purely physical value. Rubner determined this physiological value by deducting from the absolute value, the heat value of nitrogenous end-products in fæces and urine with their heats of solution. He arrived at the figure 4·015. The accepted average value is **4·1 Calories per gram of protein.**

(ii) Certain substances are excreted in combination with protein disintegration products, *e.g.* hippuric acid (protective syntheses).

(*c*) Certain substances or their disintegration products seem to be necessary constituents of fæces, *e.g.* fats and soaps (see Chap. XXVIII.).

The energy-value of all excreta must therefore be deducted from the energy intake before an energy balance can be struck.

C. Measurement of the E.V. of foods by animal calorimetry—(a) direct, (b) indirect.

It is obvious as a direct deduction from the first law of energetics that if this law holds in living as well as in non-living matter-energy transformations, the same amount of energy should be evolved from the utilisation of food inside as well as outside the body, provided always that the physical state and chemical end-products are the same in each case. If an animal could be put inside a calorimeter and given a definite amount of food, the heat evolved should, providing our hypothesis is true, be exactly the same as would be evolved in direct food calorimetry. Each gram of carbohydrate should produce 4·1 Cals., and so on. This can be put to the test in either of two ways. The first is known as direct (animal) calorimetry, and consists in accurately measuring the heat evolved by the animal under investigation. The second or indirect method is based on a knowledge of the amount of heat evolved per litre of the respiratory gases and per gram of urinary nitrogen.

(*a*) The *direct* method was first employed by Crawford (1779). His calorimeter, in principle, consisted of a double-walled box with a known amount of water between the walls. The animal was placed in the inner box for a definite time and the increase in temperature of the water noted at the end of the experiment. The method is, of course, primitive, and the veriest tyro in physics could suggest quite a host of sources of error, but on this crude instrument are based those finer implements of research which, in the hands of Benedict and his colleagues, have contributed so much to the knowledge of nutrition. Crawford found that for every 100 ozs. of oxygen used during the combustion of carbon in his calorimeter, the temperature of the water was raised 1·93° F. A live guinea-pig consuming the same amount of oxygen produced an increase of 1·73° F. This seemed sufficient evidence for him to conclude that, in each case, the heat produced was due to the conversion of pure air into fixed air, or, as we should now say, to the combination of C and O_2.

A year later, Lavoisier and Laplace published the result of

experiments which confirmed Crawford's results, and made firm the principle of indirect calorimetry. They determined the amount of ice melted by the combustion of a weighed amount of carbon (a candle) and the volume of the CO_2 evolved. A similar experiment was then tried with a guinea-pig. They found that for equal volumes of CO_2 formed, the candle yielded 25·4 cals. as against the guinea-pig's 31·8. The experiment is bristling with errors, many of which the authors realised. For instance, the respiratory and calorimetric determinations were not, as by Crawford, made simultaneously, and obviously thermal conditions were not the same. As we shall see later, cold raises the CO_2 output. If allowance is made for this and for other minor errors, the figures for candle and animal come close enough to justify the conclusion that the processes are similar, and that the source of heat in both is the combination of C and O_2.

The various sources of error due to faulty technique have been gradually eliminated, and the resultant calorimeters that bear the names of Atwater, Rosa, and Benedict and that of Williams produce results that are sufficient to convince even the most sceptical of honest observers that the oxidation of assimilated foodstuffs in the living body produces the same evolution of energy as they would if burned in the bomb calorimeter, provided the end-products are identical.

The direct method is not of such general use as the indirect. Study of the papers from the Carnegie Institute of Washington or of those from Cornell University makes clear the complexity of the machine and the intricacy of its manipulation. The cost, except for the smallest Williams' boxes, is prohibitive. The apparatus can be much simplified if the direct estimation of the energy-changes is omitted and the observer confines himself to measuring the respiratory gases and the urinary output.

(*b*) *Indirect.* As we have seen, the basis of this method also was laid by Crawford. It depends upon the following established facts :

(I) The quantity of energy liberated depends on the chemical composition of the food used.

(II) The quantity of oxygen absorbed depends also on the chemical composition of the food used ; therefore,

(III) There must be some relation between the energy evolved and the quantity of oxygen absorbed.

(IV) The three proximate principles of food differ markedly in chemical composition. (*a*) Proteins contain nitrogen, which is eliminated almost entirely in the urine. (*b*) Carbohydrates and fats differ widely in their proportion of O to C.

(V) If a determination were made of the amount of heat and

the amount of C and O_2 which corresponds to each gram of urinary nitrogen, one could, from the nitrogen excreted, calculate the heat liberated from the protein of the diet. (1 gram of urinary nitrogen = 26·51 cals.). Having deducted the protein O_2 from the total O_2 absorbed and the protein CO_2 from the total CO_2 eliminated, one arrives at the figures corresponding to the non-protein O_2 and CO_2 respectively.

(VI) Now, as we have said, carbohydrates differ from fats in their respective contents of C and O_2. Carbohydrates have the general formula $x(CH_2O)$, while fats contain less O_2 compared with their content of oxidisable matter, *e.g.* $C_{15}H_{26}O_6$. Therefore, when carbohydrates alone are used, the ratio of the volume of CO_2 eliminated to the volume of O absorbed will be 1, as may be deduced from the equation :

$$\begin{array}{c} C(H_2O) + O_2 = CO_2 + H_2O \\ 1 \text{ vol} = 1 \text{ vol} \end{array} \qquad R.Q = \frac{\text{Vol } CO_2}{\text{Vol } O_2} = \frac{1}{1} = 1.$$

Fats are compounds of glycerol, the trihydric alcohol, with organic acids of the aliphatic (fatty) homologous series. The simplest fatty acid is formic, H·COOH. The higher acids are built up by successive additions of CH_2.

TABLE III

SATURATED SERIES. $C_nH_{2n}O_2$.

E.g.	H·COOH—formic acid	CH_2O_2
	CH_3·COOH—acetic acid	$C_2H_4O_2$
	CH_3·CH_2·COOH—propionic acid	$C_3H_6O_2$
	CH_3·CH_2·CH_2COOH—butyric acid	$C_4H_8O_2$
	. . . . .	. .
	CH_3·$(CH_2)_{14}$COOH—palmitic acid	$C_{16}H_{32}O_2$
	CH_3·$(CH_2)_{15}$COOH—margaric acid	$C_{17}H_{34}O_2$
	CH_3·$(CH_2)_{16}$COOH—stearic acid	$C_{18}H_{36}O_2$

UNSATURATED SERIES. $C_nH_{2n-2}O_2$

$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$—oleic acid $C_{18}H_{34}O_2$

A glance at this list will make it clear that the amount of oxygen does not increase although the C and H are increased. This paucity of oxygen content is more marked in the fats than in the fatty acids.

$$\begin{array}{lll} \text{Palmitic Acid} & \text{Glycerol} & \text{Tri-palmitin} \\ C_{15}H_{31}CO\boxed{OH \quad H}O & & \\ C_{15}H_{31}CO\boxed{OH \quad H}O & > C_3H_5 & = C_{51}H_{98}O_6 + 3H_2O \\ C_{15}H_{31}CO\boxed{OH \quad H}O & & \end{array}$$

$$2(C_{51}H_{98}O_6) + 145O_2 = 102CO_2 + 98H_2O.$$

$$\text{Now ratio} = \frac{\text{Vol } CO_2}{\text{Vol } O_2} = \frac{102}{145} = 0{\cdot}70.$$

That is, ratio for carbohydrates is 1.
„ „ fats „ 0·7 (circa)
(Zuntz gives 0·707 as an average figure for fats.)

(VII) Values for a non-protein ratio lying between 0·7 and 1 denote the utilisation by the body of a mixture of fats and carbohydrates; the closer the ratio comes to unity the more carbohydrates are being oxidised, and *vice versâ*.

(VIII) Knowing the proportion of carbohydrate and fat in the diet, one may calculate the amount of energy set free from the two following figures:

(*a*) If carbohydrate alone is used each litre of O_2 = 5·047 Cals.
(*b*) If fat „ „ „ „ „ = 4·686 Cals.

Intermediate values may be obtained by interpolation.

(IX) The results obtained by indirect calorimetry are within 2 per cent. of results from the respiration calorimeter. In a series of twenty-two different experiments with a dog, Murlin and Lusk obtained the following results:

Indirect calorimetry . . .	2,244 Cals.
Direct	2,230 „
Difference . . .	14 „
Percentage . . .	0·6 „

(X) If the ratio is greater than 1, say, 1·5, it shows that for every three volumes of CO_2 evolved, only two volumes of oxygen are being taken from the air. This type of result is obtained either during severe exercise when an oxygen debt is being built up, or in a hibernating animal just before it begins its winter sleep, *i.e.* when fat is being formed from carbohydrate.

Further Reading

Crocker and Matthews. "Theoretical and Experimental Physical Chemistry." J. & A. Churchill.

CHAPTER IV

LIBERATION OF ENERGY

(2) THE ANIMAL AS MACHINE

"The living and the dead, things animate and inanimate, we dwellers in this world, and this world wherein we dwell, are bound alike by physical and mathematical law."
THOMPSON.

We have just seen that:

(1) Some of the radiant energy of the sun is stored by plant agency, and is ingested by the animal as food; and (2) the sum total of the energy taken in by the organism in this way can be accounted for. There is neither gain nor loss of energy in the living animal: the physical law of conservation of energy holds good.

We must consider the physics underlying the liberation of this energy. Does it follow any of the methods well known to us? Can we compare the foodstuffs to fuel and the body to a heat engine? In other words, what intermediate stage, if any, does the potential energy of, say, starch, reach before becoming apparent as animal energy? The physiological text-books are so full of references to combustion, fuel value, burning of foodstuffs, that it is natural for the student to look upon the life processes as somewhat similar to those of a steam engine. In spite of this it can be definitely said that the animal body bears little resemblance to any form of heat engine. The intermediate stage between potential and free energy is not the wasteful one of heat. In order that heat may be converted into work there must be a certain allowance for "spillage." There must also be a certain gradient of potential, that is, unless there is a certain minimum difference in temperature between the source of heat and the sink (or heat condenser), the machine will not work. (Principle of Carnot.) In 1824 Carnot determined, theoretically, the percentage of heat that any heat engine could convert into work. A theoretically perfect engine, working between the absolute temperatures T_1 and T_2, takes up Q cals. from a heat reservoir at the temperature T_1 and transforms the part W into work, then

$$W = Q\,\frac{T_1 - T_2}{T_1} \text{ (Carnot's Equation).}$$

Evidently the fraction $\frac{T_1 - T_2}{T_1}$ is that part of the Q units of heat which represents the amount of energy made available for work. That is, even under unattainably perfect conditions no more heat than $\frac{T_1 - T_2}{T_1}$ of the amount given can be converted into work. This equation gives the efficiency of the heat engine.

The most efficient steam engine yet constructed—a Nordbeg air compressor of 1,000 h.p.—converts 25 per cent. of the heat energy it receives into work. Most steam engines are only 8 to 10 per cent. efficient, *i.e.* only 8 tons out of every 100 tons of fuel burned have their energy converted into work.

TABLE IV

COMPARATIVE THERMAL EFFICIENCIES

Steam	Compound (non-condensing)	8–12 per cent.
	,, (condensing)	10–16 ,,
	Parson's turbine	15–18 ,,
Internal Combustion	Petrol (motor)	22–24 ,,
	,, (aero)	26–28 ,,
	Coal gas (stationary)	29–31 ,,
	Diesel	33–35 ,,
Combined I.C. and Steam	Still engine	41–44 ,,
Animal body		25–34 ,,

If one were to consider the animal as a heat engine, then it must operate between two temperatures. One of these temperatures we know, viz. body temperature, which is 38° C. or 273 + 38 = 311° absolute. This is the condenser or "sink" temperature. The other temperature, that of combustion, must be higher. How much higher may be calculated from the equation above.

$$\text{Efficiency} = \frac{T_1 - T_2}{T_1} = E,$$

or transposing

$$T_1 = T_2/(1 - E).$$

Suppose we take a low figure for animal efficiency, say 20 per cent. Then, substituting, we find that

$$T_1 = \frac{311}{1 - 0{\cdot}2} = \frac{311}{0{\cdot}8} = 389° \text{ absolute or } 116° \text{ C}.$$

That is to say, in order to have an efficiency of 20 per cent. with a condenser temperature of 38°, the temperature of the heat source would need to be 116° C. Experience teaches that the production of any such temperature in any tissue would cause death. Lethal temperature is somewhere about 47° C. However the animal may transform bound energy into free, it does not do so by conversion to heat as one of the stages.

It is not definitely known how the living organism is able to make use of the chemical energy of the foodstuffs. Analogy with familiar non-living machines breaks down here. An electric battery is able to transform chemical energy into kinetic energy without the middleman heat, the go-between in this case being certain unknown but ordered atomic movements. Observation shows clearly that muscle at least is not similar to an electric motor. Similarly, one can dispose of all other forms of energy-transformation used in machines to get work from bound energy.

To attempt to gain an insight into the workings of the living organism one must go back to elementary principles, and study the machine itself. The history of the foodstuffs after ingestion must be followed, and any changes they undergo must be noted. The processes of digestion, absorption and assimilation will be noted later. Meanwhile, we want to know what, in general, happens to all foods used as sources of energy. Have they, in the main, a common history? Of one point at least we may be sure, and that has been dealt with at some length in the preceding chapter, namely, that the liberation of energy in the animal is invariably followed by oxidation. Thus the amount of oxidation may be taken as a measure of the energy transformation.

Again, it has been definitely proved that all energy changes and all vital oxidations take place *in* the living cell. Physiological chemists, while unable to arrive at a definite conclusion as to the composition of the cell, are at one with the histologists in stating that the cell material is of the nature of a solution. Cell protoplasm consists of over 75 per cent. of water acting as the solvent for certain crystalloids and as the dispersed phase in various colloidal complexes. The cell comes into intimate contact with other cells, and all cell contents are not of the same chemical composition nor in the same physical state. There will, thus, arise differences in surface tension and differences in osmotic pressure. It will, therefore, be profitable for us to examine the energetics of simple solutions, then of colloid complexes, and finally to apply any relevant knowledge so gained to facts ascertained regarding cell behaviour.

We must not forget that our aim in this digression (to solution-dynamics) is to elucidate the processes by which the potential energy of foods is rendered kinetic.

Further Reading

Crocker and Matthews. "Theoretical and Experimental Physical Chemistry." J. & A. Churchill.

CHAPTER V

LIBERATION OF ENERGY

(3) ENERGY OF SUBSTANCE IN SOLUTION

"The problem of achieving perpetual motion contrary to the second law" (of thermodynamics) "is that of bringing order and direction once more into the chaotic rush of the molecules, to marshal and drill the mob so that once more they can act together to produce a common effect." SODDY.

Osmotic Pressure.

The first process that affects food is that of digestion. Digestion is merely the breaking down of the material supplied so that it can pass through the absorbing medium *in solution*. It follows (from this statement and from the physical state of the living cell) that all energy manifested by an animal comes from substances in solution. No material is of any use for energy purposes unless it is soluble, and until it is rendered soluble it cannot be absorbed and utilised.

The mere solution of a substance may so alter the state of that substance that energy is set free. (Cf. heat evolved on diluting concentrated H_2SO_4.) When a solid goes into solution it at once loses the properties characteristic of the solid state. Its particles become mobile, and all the properties dependent on regular molecular arrangement disappear. Thus the solid may be optically active or doubly refracting, and the solution quite void of these properties. The passage of the substance into solution bears some resemblance to its passage into the liquid state. A doubly-refracting crystal almost invariably loses its double refraction when it melts; and most substances which are optically active in the solid state are inactive when fused. The substance might conceivably have passed into the gaseous state. Physical chemists are agreed that this is the most probable course. They find that for *dilute* solutions, at any rate, the simple gas laws hold good.

In order to explain and correlate these gas laws and the phenomena of solution, evaporation, etc., the *Kinetic Theory* of the structure of matter has been formulated. The views that have been held regarding the constitution of solutions have been very varied, and since Thermodynamics is too general in its method of treatment to yield a complete answer to the problem, hypotheses,

guided and tested by experiment, are accepted. The following theory was first propounded by van't Hoff in 1885, and it has been improved by later physicists. It allows the Second Law of Energetics to be applied with conspicuous ease and clearness to the theoretical investigation of the quantitative relations between the properties of solutions. Matter is regarded as an aggregation of particles (molecules), each of which is perfectly elastic and structurally independent. Between them there exist spaces.

Two opposite forces are at work on molecules.

(1) *A Cohesive Force.* Newton's Law states that every portion of matter attracts every other portion of matter. The stress between them depends on the mass of the particles and the distance between them. Stress $= \frac{m_1 \times m_2}{d^2}$, where m_1 and m_2 are the masses of the particles and d the distance between them.

(2) *A Repellent Force* (Real Kinetic Energy $= \frac{1}{2}mv^2$). Every molecule is free to vibrate in a straight line within the limits of the intermolecular spaces. In a solid these spaces are small, and therefore attractive forces are predominant. If a greater kinetic energy be given to the molecules by means of heat, for instance, their mean free path will be increased at a rate corresponding to the coefficient of expansion. In a liquid the free path of the molecules becomes sufficiently long to reduce the tractative forces between the molecules to a value which is exactly balanced by the forces keeping them apart. Pellation and tractation are thus stalemate, leaving other forces, *e.g.* gravity, to determine the arrangement of the molecules in space.

If the temperature of the liquid be raised, some of the molecules will acquire sufficient velocity to burst through the surface layer and become free gas molecules. If these gas molecules move away unhindered, other molecules from the liquid will take their place, and the liquid will evaporate. If, however, the liquid is kept in a closed space, the gas molecules which leave its surface will be able to proceed no farther than the walls of this space, and rebounding from these must eventually return in the direction of the liquid. Some will strike the surface of the liquid and will be retained by it. But the molecules still continue as before to leave the surface of the liquid, so that, at one and the same time, there are molecules entering and leaving the liquid. When the pressure of the molecules leaving the surface of a liquid balances the gaseous pressure above it, a stationary state will be reached, *i.e.* the same number of molecules will be freed from the liquid as are being absorbed by it. That

pressure is the *Vapour Pressure* of the substance at that temperature. (Cf. tension of gas in solution.)

In addition to the Kinetic Theory of gases, one must assume the statement generally known as the Hypothesis of Avogadro: "*Equal volumes of different gases, at the same temperature and pressure, contain the same number of molecules.*" This proposition has been adopted as a working hypothesis, and as such has stood the test of time. It is, in fact, a necessary supplement to the Atomic Theory.

The laws governing the physical behaviour of gases are simple statements correlating *pressure, volume* and *temperature.*

(1) *Boyle's Law.* The volume of a given mass of gas varies inversely as the pressure on it, if the temperature of the gas remains constant.

$$V \sim \frac{1}{P}.$$

(2) *Charles' or Gay Lussac's Law.* The volume of a given mass of any gas varies directly as the absolute temperature if the pressure remains constant.

$$V_T = V_0 (1 + aT).$$

(3) The pressure of a given mass of any gas varies directly as the absolute temperature, provided the volume of the gas remains constant.

$$P_T = P_0 (1 + aT).$$

Any one of these laws may be deduced from the other two. The whole may be summed up in the formula

$$PV = RT,$$

where R is a constant varying only with the unit of energy used.

TABLE V

UNIT OF ENERGY.	VALUE OF R
Gram—Centimetre . . .	84,760
Joule (Volt—Coulomb) . .	8·315
Volt—Faraday . . .	$0{\cdot}8613 \times 10^4$
Litre—atmosphere . . .	0·08207
Gram—calorie . . .	1·985

The thermal constant with the *gram* calorie as unit is the one most often employed in Biophysics, and is generally taken as 2. That is, the gas equation assumes the approximate form

$$PV = 2T.$$

What is the pressure of a gas? The gas only manifests its

kinetic energy by alteration in its pressure or its volume. As has already been stated, the molecules of a gas are free to move in a straight line till they collide with another molecule or with the walls of the containing vessel. The particle will then rebound in its line of approach with a velocity equal to its original velocity, but, of course, with the opposite sign.

The pressure of a gas is due to the bombardment of the walls of the containing vessels by the molecules.

Another conclusion that may be drawn from the kinetic theory of gases is that the velocity of a gaseous particle is inversely proportional to its mass.

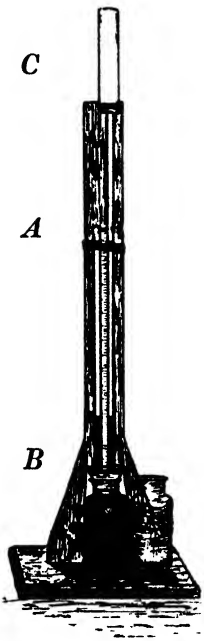


FIG. 4.—Simple diffusiometer.

This brings us to the study of gaseous diffusion. Before entering on this subject it is necessary to keep in mind Dalton's **Law of Partial Pressures**, which may be stated as follows: In a mixture of gases each gas exerts the same pressure as it would exert if it were alone present in the volume occupied by the mixture. The pressure of each gas is called its partial pressure. In other words, if several gases are brought together, each of them will be distributed through the whole space as if the other gases were entirely absent.

Gaseous diffusion takes place, and as a result there ensues in time a perfectly uniform mixture of the gases no matter what their original proportions were, and irrespective of the masses of their respective molecules. This diffusion takes place independent of gravity. A heavy gas will diffuse upwards into a lighter one.

Now, suppose we separate the two gases, *A* and *B*, by a thin porous septum such as a plug of plaster of Paris. It was observed by Graham that the pressure of the gases did not remain the same on both sides of the membrane. The pressure was greater on the side of the heavier gas. The molecules of lighter gas *B* would bombard the septum far oftener than those of the heavier gas *A*, and therefore there would be a greater chance of some of them hitting the pores and getting through and so raising the pressure in *A*.

That this is so may be very easily demonstrated by a simple piece of apparatus (Fig. 4). *C* is an unglazed earthenware cell such as is usually employed to hold the zinc rod in a Leclanché

battery. Into its mouth is fixed a rubber stopper carrying a glass tube, the lower end of which passes just through one of the holes in a similar rubber stopper of the bottle *B*. The glass tube *A* passes right through the other hole of this stopper and goes to the foot of *B*. *B* is filled with coloured water. Both stoppers must be thoroughly air-tight. If now a wide-mouthed bottle containing coal gas is inverted so as to enclose *C*, the coloured fluid will rise in *A* showing increase in pressure in *C*. If the gas used were heavier

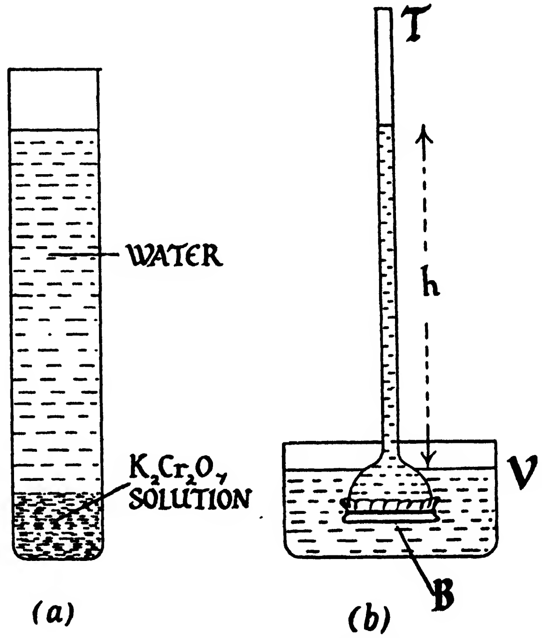


FIG. 5. (a) Liquid Diffusion. (b) Simple Osmometer.

(*a*) The coloured bichromate solution slowly diffuses into the pure water above it.

(*b*) *B* is a piece of pig's bladder tied firmly over the wide end of the thistle funnel *T*. The funnel containing the solution to be tested, *e.g.*, salt, sugar, etc., is immersed in the water in vessel *V*. After a time, the fluid in the tube will have risen a height *h* above the level of the water in *V*. The weight of this column is a measure of the osmotic pressure = $h. D. g$, where *D* is density of the solution and *g* the value of the acceleration due to gravity.

than air, negative diffusion would take place, and pressure in *A* would fall. This demonstrates the conversion of unordered molecular motion into ordered hydraulic motion capable of doing work.

The relative velocities of diffusion of any two gases through a *thin* porous septum are inversely proportional to the square roots of their densities.

This does not hold if the septum is not very thin, for then the velocity is decreased by the impact of the molecules on the inside surface of the pores.

Graham further found that it was possible to procure semi-

permeable membranes—that is, membranes which would allow free passage to one gas but not to another. What will happen in such a case ? Suppose B can pass freely through the septum while A cannot. Both gases are at 1 atmos. pressure. Then B will diffuse through the membrane, and fill up the next space as if A were not there, *i.e.* there will finally be $\frac{1}{2}$ atmos. of B on both sides. The total pressure on A will be $1\frac{1}{2}$ atmos. The excess of pressure is due to the gas A, which cannot pass through the septum. So that by taking the difference of pressure on the two sides of a semi-permeable membrane, we obtain the partial pressure of the gas to which the membrane is impermeable. (See Respiration, Chap. XXIV.)

An attempt may now be made to apply these laws (which are only absolutely true for perfect gases) to *dilute* solutions.

$$PV = RT$$

All these symbols seem applicable to a substance in solution except P. What is the pressure of a solute ? This may be determined in a way similar to the determination of gaseous pressure. If an osmometer be fitted up (Fig. 5) (Part II. p. 511) with a solution of sugar inside and water outside, in a short time the fluid inside will increase in volume and will rise in the osmometer tube developing a hydrostatic pressure. To what is this pressure due ? Obviously water (and pressure) will be transferred from a point where its pressure is high to a point where its pressure is low. In some way or other the presence of sugar (or other solute) has lowered the pressure of the water. Can this be explained by reference to the kinetic energy ? Reasoning backwards, it may be argued that the sugar acts as a drag upon the water molecules—that is, the bombardment of the membrane becomes unbalanced. The pure solvent is able to exert a greater pressure than the solution. Experiment has shown that for simple dilute solutions the magnitude of the osmotic pressure depends on the molecular weight of the substance dissolved, the amount of substance in the solute per unit volume and on the temperature of the solution. That is, osmotic pressure is controlled by just those factors which control gaseous pressure. It might be stated that *in a simple solution the osmotic pressure of a substance would be numerically equal to the gaseous pressure which the substance would exert were it a gas occupying the same volume as the solution.*

Now we have seen that the variables connected with gaseous pressure are T and V. As, according to Avogadro's hypothesis, equal volumes of gases under equal T and P contain the same number of molecules, we may state that, if T is kept constant,

P varies as the number of molecules. De Vries (1884) found that one gram-molecule of sugar dissolved in water to make up a litre, has at 0° C. an osmotic pressure of 22·4 atmos. (Practically all gases at 0° C. and 760 mm. pressure have a gram molecular volume of 22·4 litres, or conversely at 0° C. it would require a pressure of 22·4 atmos. to reduce a gram molecular volume to one litre.) De Vries, Pfeffer, and others have shown that this is true not only for sugar, but for all simple (undissociated) dilute solutions. Van't Hoff (1887) pointed out that the osmotic pressure of simple solutions is the same quantitatively as gas pressure. We have already pointed out that vapour pressure is a function of molecular activity, and may be taken as an index of the kinetic energy of the liquid. *It follows that vapour pressure varies directly with temperature.* The putting of a substance into solution lowers the vapour pressure of the solvent and, therefore, lowers its heat content. This can be deduced from the second law of energetics. From this it may be inferred that the boiling point of a liquid is always raised when a substance is dissolved in it. (These only apply to instances where the V.P. of the solute is negligible. A very volatile substance, ether, for instance, would raise the V.P. and lower the B.P.)

Correlated with these two sequelae of solution is the lowering of the freezing point. (Part II.)

The magnitudes of the osmotic pressure, lowering of the vapour pressure and freezing point, and raising of the boiling point depend in general on the number of particles in solution per unit volume. Because these magnitudes are all interrelated and are interdependent they have been named the *colligative properties* of a solution. They are definite physical quantities quite independent of semi-permeable membranes, etc. The membranes make osmotic pressure apparent.

Osmotic pressure is of considerable magnitude. We have seen that a gram-molecular solution has an osmotic pressure of 22·4 atmos., *i.e.* 303 lbs. per sq. inch. The ordinary dilute laboratory reagents develop a pressure of about 50 atmos. In a plant, root pressure has been estimated at about 60 feet of water.

If, however, one were to tabulate the osmotic pressure of gram-molecular solutions of all substances, one would find that solutions could be divided into three great classes.

Class 1.	O.P. Approximately	22·4 atmos.,	*e.g.* Sugar.
,, 2.	O.P. Decidedly greater than	22·4 ,,	*e.g.* Salt.
,, 3.	O.P. ,, less ,,	22·4 ,,	*e.g.* Starch.

The first class have been termed simple (undissociated) solutions.

According to the kinetic theory the second and third classes have a larger or smaller number of particles in solution than theory warrants.

Where have the extra particles in Class 2 come from? Has the molecule divided? If one compares the osmotic pressure of cane sugar and sodium chloride in gram-molecular solution, one finds them (roughly) as 1 : 2. How can this be explained? In 1887 Arrhenius propounded his dissociation theory, which has since been proved, amplified and universally accepted. According to this theory, some of the molecules of certain salts when dissolved in water split up or dissociate into their constituent ions. An ion is an atom or a sub-molecular group charged with electricity and attached to certain water molecules.

For example,

$$\begin{aligned} \text{NaCl (solid)} + \text{aq} &= \text{cat-ion} + \text{an-ion} \\ &= \text{Na} + \text{Cl} \\ &= (\text{Na}(\text{H}_2\text{O})x) + (\text{Cl}(\text{H}_2\text{O})y). \end{aligned}$$

It is the presence of these ions which gives a solution the power of conducting electricity, and so any substance which dissociates, *i.e.* becomes ionised on going into solution, is said to be an electrolyte (Chap. VII.).

It is worthy of note that electrical conductivity is not a property either of the solvent or of the solute, but of the solution. (Part II.)

All electrolytes are not dissociated to the same extent. A salt of either a strong acid or a strong base requires the addition of comparatively little water completely to convert all its molecules into ions. On the other hand, a weak acid or base is difficult to dissociate. If the gram-molecular weight of an electrolyte be dissolved in a litre of water a certain fraction of the molecules will split into ions. This fraction is the degree of ionisation of the electrolyte at this concentration. The degree of ionisation may be determined by estimating the amount of resistance of the solution to a small electric current (conductivity method), or it may be approximately calculated from the lowering of the freeezing point. (For univalent strong electrolytes at concentrations not exceeding 0·1 molar, the error of this determination is in most cases between 1 and 4 per cent.)

One may note in passing that the ions of many electrolytes possess the property of uniting with other ions, or even with non-electrolytes in solution, to form complex ions. For example, ions cannot normally remain free in aqueous solution, but become hydrated. A hydrated ion is sometimes termed an **ionic micelle.**

In solutions of the third class, the O.P. and other colligative properties point to a reduction in the number of particles in solution. A clubbing of molecules has taken place. Because most of the substances that compose this group have a somewhat gluey consistency, Graham called them colloids (Gr. κόλλη = glue). The physics of colloidal complexes will be dealt with in a separate chapter. Here we merely wish to draw attention to their low osmotic pressure. Colloid substances may be converted into non-colloid or crystalloid derivatives, and so liberate energy, *e.g.* starch, a colloid having a very low osmotic pressure, may be readily hydrolysed to maltose, which is a crystalloid—non-electrolyte, having a molal O.P. (*i.e.* belongs to Class 1). Glucose, which similarly has a molal O.P., may be stored in the liver as glycogen—a colloid, which again readily undergoes hydrolysis (to a crystalloid). (See Chap. VIII., Colloids.)

Further Reading

Findlay. "Osmotic Pressure." Longmans, Green & Co.

CHAPTER VI

LIBERATION OF ENERGY

(4) SURFACE ENERGY

"This Phaenomenon proceeds from a propriety which belongs to all kinds of fluid Bodies more or less, and is caused by the Incongruity of the Ambient and included Fluid, which so acts and modulates each other, that they acquire, as neer as is possible, a *spherical* or *globular* form." HOOKE, 1665.

(See also Chap. XIV. Muscular Contraction; XV. and XVI. Secretion and Excretion; XVIII. Nerve Conduction; XXVII. Respiration.)

Observation shows that the surface differs markedly in physical state from the interior of a liquid. The surface between air and water, the air-water interface as it may be called, is able to withstand the application of a considerable distorting force without rupture. This fact may be demonstrated in a variety of ways, *e.g.*,

(i) A sewing needle carefully placed on such a surface causes the water to bend to accommodate its weight (Fig. 6).

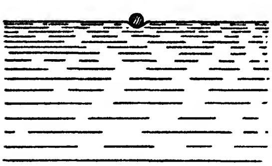


FIG. 6.—To show the depression of the surface of water when a needle is floated on it.

(ii) A cold tea-spoon or other object lifted from the surface of a cold liquid will stretch some of the surface film adherent to it for quite a considerable distance (Fig. 7). If the fluid and spoon are warm the film will break more readily. Increase of temperature lowers surface cohesion.

(iii) Water creeps up the sides of beakers, is soaked up by blotting paper, sponges, charcoal, capillary tubes, etc., against gravity.

(iv) Water beetles walk safely over the surface of water, and large heavy *clams* may suspend themselves from filaments anchored to the under-surface of the water in aquaria. These examples show that the film of water molecules at the surface possess a remarkable tensile quality, surface energy, surface tension or cohesion which in the case of a water-platinum interface is about 73 dynes per cm. at 20° C.

How Measured.—There are four methods in common use for the determination of either the absolute surface tension or its value relative to water.

1. *By means of the torsion balance* (Fig. 8) the force necessary to lift a ring, plate or straight wire from the fluid-air interface is determined.

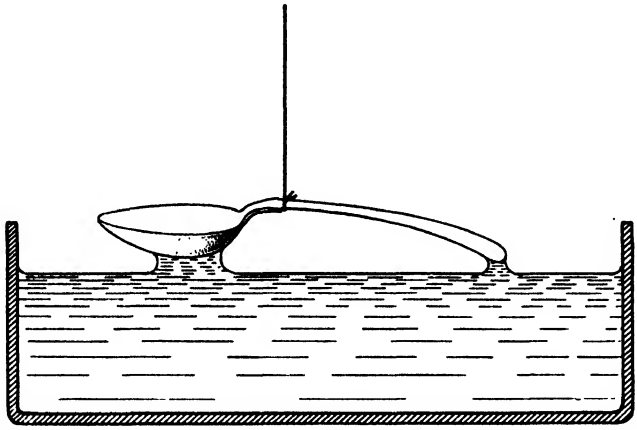


FIG. 7.

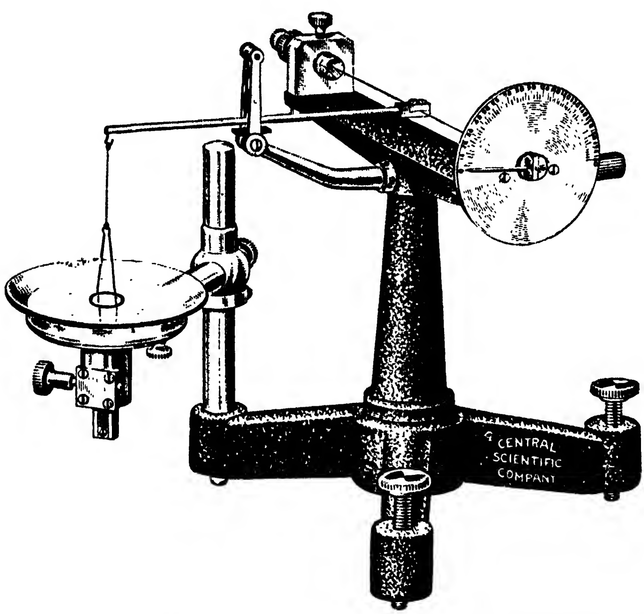


FIG. 8.—Du Nouy's Surface Tension Apparatus. One cubic centimetre of the liquid to be tested is placed in the watch-glass. The force required to pull a platinum ring from the fluid surface is applied by twisting a wire The amount of torsion, and hence the value of the surface tension, is given on the graduated dial.

2. *Drop-weight Method* (Part II., p. 517). The weight of a drop falling by gravity *under standard conditions* (size of dropping surface, etc.) gives a measure of the tension on the surface of the drop.

3. *Capillary Rise Method.* The height to which a liquid will rise in a capillary tube held vertically on the surface is an inverse measure of the surface tension of the liquid. (Part II., p. 518.)

4. *Air Bubble Method.* A value for the surface tension of a liquid may be obtained by measuring the amount of force necessary to blow a bubble of air into it from a capillary tube.

Ageing of Surfaces.—All these methods require the observance of standard conditions. For instance, *it takes time* for a new surface to attain its normal tension after it has been disturbed by the placing of a ring on it, by the formation of a new drop, or by forcing it out of a tube by a bubble of gas. In fact, the adjustment of a surface may go on for hours, though the maximum change takes place very rapidly. After the elapse of a minute an approximately normal value for surface tension may be obtained. Again, the temperature at which the measurements are carried out is important—the higher the temperature the lower the tension developed, till at the critical temperature it reaches a minimum value. That is, surface tension is a phenomenon with a *negative temperature coefficient.*

These facts may be explained by reference to the forces which act on all molecules—in solids, liquids and gases.

Two forces act on molecules :

(*a*) A repellent force—kinetic, revealed in vapour tension, etc.

(*b*) A cohesive or attractive force—Newton's " Gravity."

The latter gives rise within the liquid to intrinsic pressure, whose magnitude we have no direct means of measuring, and whose energy we cannot utilise—because the various tractative forces acting on each and every molecule *within* the liquid neutralise one another. The attractions, except on the surface layer, are uniform and cancel out. Consider a single internal molecule. The tractative forces acting on it in any plane may be resolved into four components acting cyclically at right angles to one another. It is obvious that these forces are paired. That at twelve o'clock is equal to and opposite to that at six, and therefore ineffective. Similarly, the eastwards pull at three o'clock is neutralised by the westwards pull at nine. In the surface layer, matters are different. One component, that is the force pulling downwards, has no opposing upward force to stabilise the molecule. There is, therefore, a state of strain in the surface area.

Orientation on Surfaces.—As the result of this state of strain the molecules at the surface are arranged more regularly than those in the body of the liquid. One may consider the internal molecules of water as lying at random with their long axes in no particular plane, while at the surface the long axes are practically parallel to

one another and at right angles to the surface. The surface layer or layers, because of their orderly arrangement, will, therefore, have a larger number of molecules per unit area than the interior of the liquid with its higgledy-piggledy arrangement of molecules (Fig. 9). That is, the surface will tend to decrease under the uncompensated Newtonian attraction of under surface molecule for surface molecule, and the tension so produced will cause the orientation of the molecules at the surface, the effect on any individual molecule being determined by the extent to which the surfaces of that molecule (on which equal forces are acting)

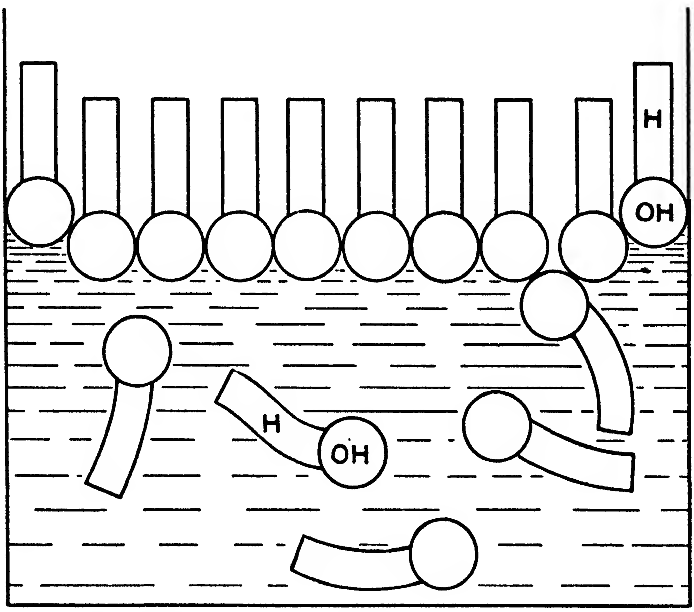


FIG. 9.—To show how the surface of water differs from the interior in the orientation of the molecules. A similar orientation occurs at the glass-water interfaces.

deviate in shape from spheres drawn about the centre of mass. Various experiments have been devised to determine to what extent this orientation is transmitted to molecules lying at a distance from the surface. Hardy's proof that the oriented layer may be several molecules thick is both simple and unequivocal. He allowed the fluid under test to be drawn in by capillarity between, say, a microscope slide and a weighted cover-slip. The force exerted is sufficient to lift the cover-slip and its weight from their bed. Every molecule of fluid drawn in must, by the fact that it is drawn in, be under the influence of the glass surfaces. The fluid is now frozen, and the cover-slip broken away. The layer of solidified fluid left is quite visible and is capable of measure-

ment in depth, in tensile strength and in resistance to shear. This experiment indicates that the thickness of the layer under the influence of surface forces is at least of the order of 1,000 molecules, its value depending on the eccentricity of the equipotential surfaces of the molecules. The greater the eccentricity of the fields of force about the molecules (*i.e.* the greater their polarity) the thicker will be the oriented layer.

Utilisation of Surface Energy.—As we have seen, the energy developed at the surface is considerable. If some means could be devised whereby this energy could be freed from the surface, or, what comes to the same thing, if its intensity could be altered, then it might be utilised to bring about changes in matter. Such a potential power might have considerable significance in physiology. The tissues of which organs consist and the cells that compose the tissues abound in surfaces or interfaces where one liquid phase subjoins another similar or dissimilar phase. Alterations in surface tension, quite apart from gross energy changes, play a large part in physiological processes, as we shall see later.

Alterations in Surface Tension.

A. Pure Liquids.—(1) Whatever alters the intrinsic energy of the liquid will produce a corresponding alteration of the energy on the surface. The attractive force between molecules of a liquid (or gas) varies from absolute zero to the critical temperature directly as a *constant* and inversely as the *square of the distance between* the molecules. That is, increase of temperature will tend to lower surface tension. In other words, *surface tension has a negative temperature coefficient.*

This means of varying surface energy is not of great interest to the biologist, as it implies alterations of temperature which to be significant have to be considerably more than is compatible with life.

(2) The electrical state of a surface layer is of interest in this connection. Electrons accumulate on the aqueous side of a water-air interface and they tend to cause the surface to expand. If they are increased in number their mutually repellent power will actually overcome the contracting power of Newtonian gravity, and the surface will increase in curvature, *i.e.*, expand, and the surface tension be lowered. Examine the surface, for instance, of a globule of mercury in water. The water molecules on this surface are arranged with their polar ends, *i.e.*, OH radicle, in the water and the H^+ ion pressed against the mercury. A double electrical layer thus exists. The metal, by virtue of the closely adherent H^+ ions, takes on a positive charge on its surface, while just external

to this is a layer of negatively charged hydroxyls. If these two opposing charges balance one another, the shape of the globule will be defined by surface forces alone, *i.e.*, the surface will be reduced to a minimum and the globule will appear spherical, except, of course, at the mercury-glass interface. Now increase the concentration of positive ions in the water by adding a drop of sulphuric acid to it, and so reducing the number of *effective* electrons on the surface of the water adjacent to the mercury, and the balance between protons and electrons is disturbed in favour of the former.

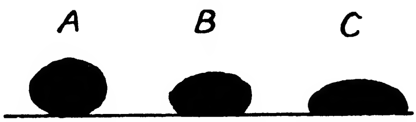


FIG. 10.—Form assumed by mercury globules under different electrical states. *B* is a mercury globule immersed in dilute H_2SO_4. It assumes a more spherical form *A* when connected with the negative pole of a battery, while connection to the positive pole reduces surface tension as shown at *C*.

The surface forces are, therefore, partially overcome by the electrostatic repulsion of similarly charged surface molecules and the globule will flatten out (Fig. 10, B). Increasing the positive charge on the mercury by connecting it to the positive pole of a battery and immersing the lead from the negative element in the acidulated water will cause the surface forces to be still further reduced (Fig. 10, C). The reverse effect occurs when the direction of the electrical current is reversed. A very neat demonstration of this, due to Ostwald, may be given by actually making the mercury one of the elements in a galvanic couple. (Part II., p. 517.)

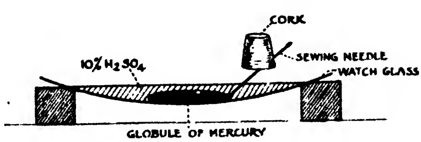
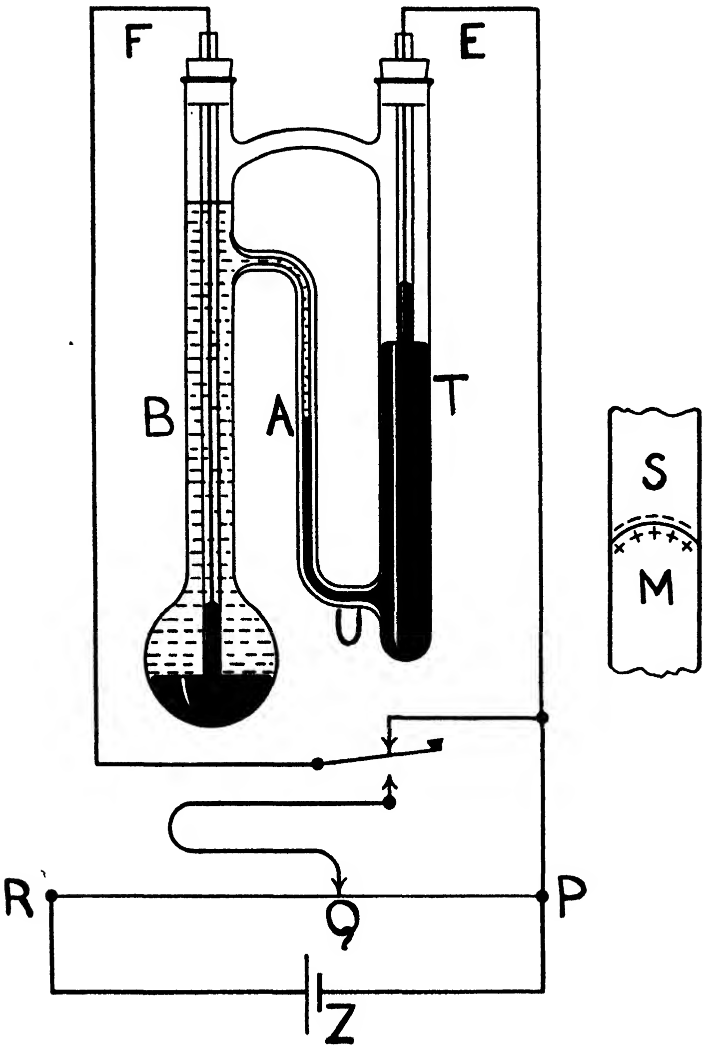


FIG. 11.—Mercury "Heart."

In this experiment (Fig. 11) the mercury forms the positive element and the carbon in the steel needle the negative element. When connection is made between the + and — substances by placing the needle so that it just touches the mercury the charge on the mercury surface is decreased, the globule more nearly assumes the spherical form and so breaks connection with the needle. This allows the positive charge on the metal to accumulate, lower the surface tension, and so again make contact. (To prevent the cessation of this rhythmic movement by polarisation, a little potassium bichromate is added to the fluid.)

Capillary Electrometer.—Lippman made use of the electrical alteration of surface tension in his capillary electrometer which



(A) (B)

FIG. 12.—Diagram of Capillary Electrometer (Crocker and Matthews).

12A.—*T*, the reservoir of mercury containing the insulated electrode *E*, communicates by *U* with the capillary *A*. Above the mercury in *A*, and filling the upper part of *B*, is dilute sulphuric acid. *F*, the other electrode, passes into the mercury at the bottom of *B* and *F* is kept out of contact with the acid. When not in use *F* and *E* are short circuited by a key.

B represents the surface of the mercury *M* in contact with the acid *S*.

consists essentially of a capillary tube containing mercury and dipping into dilute sulphuric acid (Part II., p. 520 and Fig., 12). The mercury contains one leading in wire from a source of potential difference, while the other lead is taken to a small amount of

mercury in the bottom of the vessel holding the acid. The position of the meniscus in the capillary is observed through a microscope or is projected on a screen. A very slight increase in the charge on the mercury-acid interface will lead to a movement of the mercury *in the direction of the current.* That is, if the mercury holds the positive lead the meniscus will fall (S.T. lowered); conversely, if the negative pole from the battery is attached to the capillary, the meniscus will rise (S.T. raised). *The extent of movement depends on the difference of potential developed* (Figs. 38 and 43).

B. Solutions.—The surface layers of electrons may be increased or decreased by the addition of solutes which, by altering the intrinsic energy of the solution, cause a redistribution of energy, and so new surface relationships are produced. The material dissolved in a fluid is not dispersed regularly, but is generally found more concentrated at surfaces. According to the Gibbs-Thomson principle, those solutes which tend to lower surface tension are found at the surface, while those which raise surface tension are least concentrated at the surface, *i.e.*, "*The concentration throughout a fluid tends to be so adjusted as to reduce the energy at every point in it to a minimum.*" Very few substances raise surface tension, and that to a very slight extent. Strongly dissociated inorganic salts have very little effect either way, but are usually found *adsorbed* to surfaces. This fact is attributed not to any property of the salts, but to the unsatisfied valencies existing at any surface wetted by the solvent. (See also Adsorption, p. 53.)

C. Capillary Active Substances.—The surface tension of water is markedly diminished by certain organic substances with long carbon chains. The longer the carbon chain, and the smaller the number of decidedly *electro-positive* and (particularly) *electro-negative* groups (such as $-$OH and $-$COOH) they possess, the more powerful is their action. These substances, which are also distinguished by a high degree of *adsorbability* (*q.v.*) and by powerful biological actions (cf. anæsthetics, etc.), are called *capillary active* from their effect in lowering the level of water in a capillary tube. They have also a low surface tension themselves and are of interest because of the orientation of their molecules on the surface of water. Typical physiological capillary active substances are found in saliva, bile, blood and milk. These substances all have in their carbon chain a radicle which is particularly soluble in water, *e.g.*, carboxyl (COOH), hydroxyl (OH), $COOCH_3$ or CN. Apart from this *polar radicle*, the remainder of the molecule is insoluble or markedly less soluble in the solvent. They are, therefore, arranged like a fisherman's floats with their soluble polar ends in the water and the rest of the molecule standing vertically out of the water

(Fig. 13). Fatty acids (Experiment 11) lower the surface tension of water because the unsatisfied valencies at the surface are satisfied by the soluble COOH group, while the insoluble paraffin portion remains out of the water. That is, the fatty acid or other *capillary active substance* goes to the surface because that portion of the molecule which does not wet tends to leave the water, but is anchored to the water by the polar group. If there is sufficient of the substance present to cover the surface with a layer at least one molecule thick, then the surface tension will be decreased. Compare this surface orientation with Experiment 18, where small bits of paper coated on one side with lamp-black or printer's ink orient themselves on the interface between paraffin oil and water so that the blackened sides are turned towards the oil.

Adam has found that the gathering together of fats or fatty acids

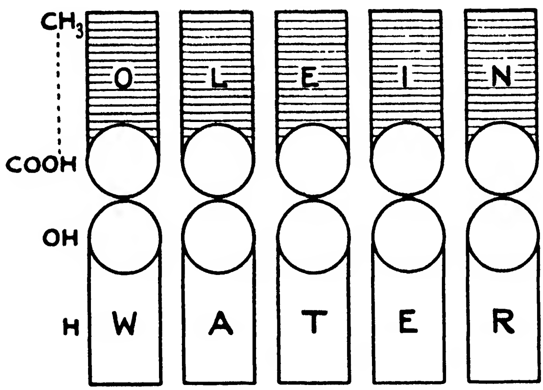


FIG. 13.—To show how an oil like Olein containing Oleic Acid has its molecules oriented on the surface of water, COOH radicle attracted to OH radicle.

in an orderly fashion like this on a surface produces changes in the physical state of the substances so oriented. For instance, palmitic acid at room temperature is solid, but if placed on the surface of water at the same temperature is clearly liquid. Further, the surface layer is able to withstand a considerable lateral force without buckling. When the packing force is applied the molecules on the surface fit into one another like spoons in a box and so allow of more molecules per unit surface. If the polar portion is bulky the molecules pack into a curved film having the hydrophobic portion concave and the hydrophilic polar part convex. Not until something like 100 atmos. pressure has been applied laterally will buckling of the film occur. Some substances go completely out of solution when so adsorbed on a surface. The molecules of albumin, for instance, when oriented on a surface adhere together and form an irreversible gel, *i.e.*, they coagulate. *This adherence*

to one another is a common attribute of all long molecules capable of forming a closely packed surface film. (See Chap. XI., Membranes.)

Adsorption.—It has been found that surfaces of solids and liquids exert an attraction for gases. It is very difficult, for instance, completely to remove air from the surface of a glass container. Repeated evacuation is necessary. The amount of gas so adsorbed varies with the nature of the surface, and of the gas, as well as with the pressure, and inversely with the temperature, and is a *reversible* process. That is, the processes of adsorption and de-adsorption will proceed together till a condition of equilibrium has been reached which will remain undisturbed as long as the temperature and pressure of the system remain unaltered. (Principle of Le Chatelier.) Charcoal, which has an enormous surface area per unit volume, will adsorb large quantities of gases, *e.g.*, 1 c.c. of coconut charcoal at 0° C. will adsorb 2 c.c. of helium, 4 c.c. of hydrogen, 15 c.c. of nitrogen, 18 c.c. of oxygen, 75 c.c. of ethylene, 171 c.c. of ammonia. Use is made of this property in the preparation of high vacua, in the manufacture of "gas masks," and for the removal of foul gases, etc.

At a liquid-gas, liquid-liquid, or at a liquid-solid interface adsorption readily occurs and may easily be demonstrated by the use of coloured solutes. (Part II., Experiments 18/43 (*c*).) If we increase the surface of a liquid by the introduction of a finely divided gas, immiscible liquid, or solid, we may be able completely to remove substances in solution. Here we find that the physical and chemical nature of the adsorbing surface and of the adsorbed material are of prime importance. Some adsorbents are almost omniverous, *e.g.*, charcoal in aqueous solutions, others, like kaolin and ferric hydroxide, which have the greatest conceivable *specific surface* (see p. 72), will only adsorb certain types of solute, due to the sign of the electric charge which they develop in contact with water. Most of them, like kaolin, become negative, but a very few, like ferric hydroxide and hæmoglobin, become positive. The former adsorbent will, therefore, fix electro-positive dyes such as the "basic" dye methylene blue and the latter electro-negative dyes.

Practically all dyes are salts of a coloured and a colourless ion. If the organic *base*, united generally with hydrochloric acid is coloured the dye is termed *basic*. A coloured organic *acid*, may unite with a colourless inorganic base to give an *acid dye*. The former because of its hydrion is *electro-positive*, the latter is *electro-negative* on account of its natrion.

Chemical forces come into play in some adsorptions, for example, the adsorption of that class of substances termed "capillary or surface active" is determined not by the extent or the *physical*

nature of the surfaces to which they are exposed, but by the *chemical* nature of the surfaces. Kaolin and ferric hydroxide will not adsorb even a trace of tri-butyrin or acetone, or of any of the higher alcohols, or, in fact, of any capillary active substance.

The amount of any solute adsorbed depends on its concentration and temperature, as well as on the nature of the force causing the adsorption. If the volume and temperature of a solution are kept constant while the quantities of adsorbent and of material to be adsorbed are varied, it is found that when *adsorption equilibrium* has been established, the relation between the amount of substance absorbed (x) and its concentration unabsorbed in solution (c) is given by Freundlich's equation :

$$\frac{x}{m} = kc^n$$

where m = weight of adsorbent present,
k is a constant depending on the nature of the adsorbent and is the amount adsorbed when $c = 1$
and n is a constant depending on the nature of the adsorbed substance.
(The value of n is usually about 0·5.)

Four further points are of interest :

(1) *The rapidity with which adsorption takes place.*

(2) *The reversible nature of adsorptions of the purely physical type.* If definite amounts of adsorbent and adsorbable substances are allowed to attain equilibrium and then the concentration of either of them altered, a new equilibrium point will be reached. For example, if after charcoal has removed a certain quantity of a solute from a solution, the solution be diluted with an equal quantity of water, so reducing the concentration of the charcoal and of the unabsorbed material by half, then some of the material will be given up by the charcoal to the solution. The final concentrations will be the same as if one had started with half the quantity of charcoal.

(3) *The phenomenon of adsorption-displacement.* If two or more adsorbable substances are present in solution and *no chemical action* takes place only *one* substance will be adsorbed—the more sparingly adsorbable substances will remain in solution. On the other hand, if a substance A is adsorbed and then B which is more powerfully adsorbed is added to the solution, A will be *completely* expelled from the surface. Capillary active substances, by powerfully lowering surface tension, cause the expulsion of all less active substances from a charcoal-water interface. (See Chap. XI., Adsorption Membranes, and Part II., Experiments 18 and 20.)

(4) *The minute nature of the quantities of solute which may be removed from solution.* A 100 c.c. of a 1 in 10,000 solution of crystal violet or eosin is completely decolorised by 1 gram of charcoal in less than 2 minutes.

The biological significance of adsorptions will be considered in later chapters.

D. Suspensions.—When very tiny particles of insoluble matter, *e.g.*, gold, carbon, etc., are dispersed through water, a large increase of surface is produced and the physical properties of the liquid become so remarkable that a separate branch of science with a technique of its own has been evolved to study them. They will be dealt with in a subsequent chapter.

A special case of the multiplication of surface may be considered now. A piece of charcoal has not only an external surface, but its interior is ramified by a series of larger channels or macropores (mean diameter 12μ) and tiny capillary bores or micropores (mean diameter 10 $\mu\mu$). When gas-free charcoal is immersed in water it soaks up the fluid like a sponge, but takes in more water than its volume would appear to justify. That is, some of the imbibed water is compressed. This compression occurs in the micropores with, of course, the evolution of heat. The net heat of adsorption is closely proportional to the heat of compression under high pressures. One gram of charcoal (not evacuated) immersed in water gives off something like 18·5 gram cals. The molecules of water in the micropores are arranged in parallel rows, closely packed together, and so occupy less volume than either the molecules in the macropores or in bulk.

Tissues abound in potential micropores and in interfaces, and, therefore, we would expect that surface forces would play an important part (*a*) in the structure of living matter, and (*b*) in maintaining a balance between free and potential energy.

Further Reading

Willows and Hatschek. "Surface Tension and Surface Energy." J. and A. Churchill.

SECTION II.: CELLULAR MECHANICS

CHAPTER VII

IONISATION

IONS—THE WORKMEN OF THE CELL

"Many things move me to suspect that everything [natural as well as mechanical] depends upon certain forces, in virtue of Which the particles of bodies, through forces not yet understood, are either impelled together . . . , or are repelled and recede from one another." NEWTON.

THIS subject has been alluded to in connection with abnormal osmotic pressures (Chap. V., p. 42), where it was pointed out that electrolytes, on going into solution, were more or less dissociated into their constituent ions. The extent to which an electrolyte is thus dissociated determines whether it is a strong or a weak electrolyte. Inorganic acids and bases and their salts are almost completely dissociated in solution, the dissociation increasing with dilution until, of course, complete dissociation is reached. Organic acids and bases, *as a rule*, are dissociated with difficulty, complete dissociation being reached only at great dilutions. There are exceptions--some organic bases are just as well ionised as the strongest inorganic bases. Guanidin salts, for instance, have dissociation values lying between sodium and barium salts. Salts formed of a weakly dissociated acid and a strongly dissociated base or of a weak base and a strong acid have dissociation values intermediate to those of their constituents.

There are two outstanding points of interest about ions.

1. Ions are always electrically charged, the "metal" ion having a positive and the "acid" ion a negative charge. (*The former*, in Faraday's terminology, *is called a cation and the latter an anion.*)

2. Ions are never free, but are always hydrated.

Electrical Charge.—It is obvious, if two electrodes from a source of supply be dipped into a solution containing ions, that in virtue of their charge the anions (negative ions) will be attracted towards the anode (positive electrode) and the cations (positive ions) will be drawn towards the cathode (negative electrode).

Such a solution will therefore conduct electricity, and further, its efficiency as a conductor will depend on the number of ions present, *i.e.* on the dissociation of the solute.

This provides the basis on which the method for estimating the concentration of ions has been devised. In doing this the electrical resistance, in ohms, is measured. Conductivity is the reciprocal of resistance (Part II., p. 522).

Relative Speed.—Another factor must be taken into account. We have seen that ions do not all move at the same rate. The rate depends on the atomic weight of the ions, the degree of hydration and the influence of other ions. (Ions by their electric charge influence one another.) Under similar conditions, each ion moves at a constant rate.

That the rate is slow is shown by passing an electric current through a solution containing coloured ions at one electrode and noting the time they take to reach a similar concentration at the other electrode. Kohlrausch determined the relative speed of ions at 18° C. and for a constant potential gradient found as follows :

TABLE VI

Cations +			Anions −		
Ion.	Atomic Wt.	Relative Speed.	Ion.	Atomic Wt.	Relative Speed.
H	1	318	OH	17	174
K	39	64·6	$\frac{1}{2}SO_4$	48	68
NH_4	18	64·4	Br	80	67
$\frac{1}{2}Ba$	68·5	55	I	127	66·5
$\frac{1}{2}Sr$	43·7	51	Cl	35·5	65·5
$\frac{1}{2}Ca$	20	51	$\frac{1}{2}C_2O_4$	44	63
$\frac{1}{2}Mg$	12	45	CH_3COO	59	33·7
Na	23	43·5			
Li	7	33·4			

Bredig has pointed out that with organic ions the longer the carbon chain, the less the speed. The decrease in speed is, however, comparatively slight.

Hydration.—Consideration of the numerical values of the relative ionic rates gives a means for calculating the hydration of the various ions. It may be taken for granted that the speed of ions apart from hydration is proportional to their mass, *e.g.* potassium and chlorine have approximately similar atomic weights and similar speeds. Any variation from this proportion is usually attributed to the different hydration of the ions. It is generally

conceded that, as stated above, all ions are hydrated. Therefore potassium and chlorine must be hydrated to almost the same extent. Bousfield has shown that 9 water molecules are attached to both ions of potassium chloride when completely dissociated. Now, as the speed of K to Cl is as $\frac{64{\cdot}6}{39} : \frac{65{\cdot}5}{35{\cdot}5}$, *i.e.* as 16 : 19, almost as 4 : 5, it may be considered that K has 4 and Cl 5 water molecules per ion.

In the group of alkali metals tabulated above it will be seen that the lightest metal, lithium, furnishes the most sluggish ion of the three, and conversely, the most mobile ion is that of the heaviest metal, potassium, sodium being intermediate both in atomic weight and in speed. This is supposed to mean that lithium is more heavily hydrated than sodium, and sodium more than potassium. The number of molecules of water combined with their chlorides when completely dissociated is respectively, 21, 13 and 9. If the 5 molecules of water which form an envelope for the chlor-ion be subtracted from the total, lithium is found to be hydrated to the extent of 16 and sodium to 8 molecules.

Effect of Temperature.

Increase in temperature according to the kinetic theory and laws of energy will increase the speed of ions, provided, of course, that dissociation is complete. Partially dissociated salts are more completely ionised by increase in temperature. For equal increment of temperature, different ions increase in speed according to their degree of hydration. The more highly hydrated the ion, the greater is its temperature coefficient. This is explicable on the hypothesis that a rise of temperature will favour the disruption of hydrate-complexes and decrease the size of the ion, and so reduce the frictional resistance to its passage through the fluid.

When dealing with surface tension (p. 48), the Helmholtzian double layer or surface electrical charge was mentioned. This may now be attributed to the different ionic speeds. Whichever of the two ions has the greater mobility will get into the surface layer and, of course, will carry its charge with it. This will cause the mobilisation on the immediately opposite "side" of the surface of oppositely charged ions.

There exists an enormous electrostatic attraction between ions of opposite sign. The introduction of other electrolytes into a solution may therefore alter not only the rate of migration of the original ions but the nature of the surface charge. The addition of HCl to a solution of KCl would increase the diffusion potential that would be produced at the boundary between solutions of KCl

at different concentrations; the more HCl present, the greater the diffusion potential. This is due, of course, to the relatively greater speed of the hydrogen ion. The K ions move at about the same speed as the Cl ions, while the H ions move about five times as fast. The boundary surface previously charged negatively with a low E.M.F. would take on a positive charge with a higher E.M.F. It is imperative to note that unless the electrostatic force mutually exerted between anion and cation is overcome, these ions though separated will never be far apart.

In ordinary solutions the "metal" ion, no matter what its relative speed, cannot be separated from its "acid" ion by mere diffusion. The disturbance of electrical equilibrium caused by the introduction of electrodes into the solution will produce a separation of the salt into metal and acid.

Now, if there exist equal and opposite charges on an- and cat-ions, tending to draw them together, why, in the first instance, did they separate, and what keeps them apart? This brings us to the discussion of the **dielectric constant.** To put a name on a thing or on a process does not explain it. Neither is it sufficient to say that the dielectric constant or specific inductive capacity of any medium is a measure of the capacity of that medium to act as a dielectric (non-conducting) substance of an electric condenser. The higher the value of the constant, the greater is the value of the condenser.

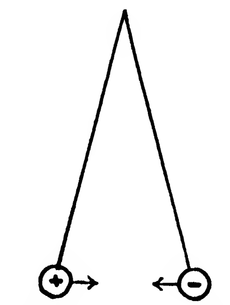


FIG. 14.—Model of anion and cation. Two pith balls suspended by silk threads attract one another if carrying opposite charges. When the charges are of the same sign, the balls diverge, *i.e.*, repel one another.

According to the **electron theory,** an atom is composed of protons and electrons. Electrons are all similar, and are supposed to be not sensible matter, but the smallest possible unit of negative electricity. Atoms of different substances owe their different qualities to the varying number of electrons they contain and to the diversity of their arrangement. These electrons are supposed to exercise an obstructing influence on the passage of an electric charge due to their tendency to move in the direction opposite to the direction of the current. The larger the number of the electrons, therefore, the greater the obstruction. Now it can be shown that when two small electrically charged bodies (charges e and e' respectively) are immersed in a medium at a distance r apart, the force they exert on each other equals $\frac{ee'}{Kr^2}$, where K is a constant for the medium and is known as the dielectric constant.

It is a measure of the obstruction produced as described above, *i.e. it measures the capacity of the medium to act as an insulator.* When the distance r between the two charged bodies is increased so that Kr^2 is very large compared with ee', the force $\frac{ee'}{Kr^2}$ becomes negligible, and the two bodies will cease to attract (if of opposite signs) or repel one another (if of the same sign). Suppose this happens at a distance r_1 in a medium with a dielectric constant K_1, and at a distance r_2 when the medium has a dielectric constant of K_2,

then
$$\frac{ee'}{K_1 r_1^2} = \frac{ee'}{K_2 r_2^2} = 0$$

and as the charges e and e' are obviously the same in both instances,

then
$$K_1 r_1^2 = K_2 r_2^2 \text{ and } r_2 = r_1 \sqrt{\frac{K_1}{K_2}}.$$

From the following Table (VII.) it may be seen that air is arbitrarily taken as having unit dielectric constant, and on this basis water has a dielectric constant of 81·7.

TABLE VII

Air	=1·0	Water	=81·7
Co_2	=1·0004	Alcohol	=25·0
H	=0·9997	Formaldehyde	=84·0
		Acetic Acid	= 6·46
		Vaseline, Paraffin	= 2·2
		Liquid Fat	=3·3·2

If the media r_1 and r_2 referred to above are *air* and *water* respectively, and substituting values in the equation for r_2, we have

$$r_2 = r_1 \sqrt{\frac{1}{81 \cdot 7}} = \frac{r_1}{9} \text{ approx.}$$

That is, the distance between charged ions in water may be nine times less than in air without the one ion exerting an appreciable influence on the other. Thus, when the molecules have been split into their constituent ions, the high dielectric constant of water lowers the probability of their recombination to such an extent that the solution is stable in this dissociated form.

This does not, however, describe how the splitting of the molecules into ions is brought about, nor why some substances are easily and almost completely dissociated at a certain dilution, while others under the same conditions undergo an almost negligible dissociation. Many solutes dissolve in water to give highly dissociated solutions without any great change in the sum total of

the energy content of the reacting substances. Yet, out of solution, molecules can only be resolved into their atoms or dry gases ionised, by the application of considerable external force. The latter phenomenon has been much studied of late years, especially in connection with the passage of X-rays and ultra-violet rays, and it has been found to depend on the *frequency* of the incident radiation. The former rays knock electrons off the molecules of the oxygen and nitrogen of air 1,000 times more efficiently than the latter rays because their frequency is 1,000 times as great. That is, *the energy of escape of electrons from gases is an accurately linear function of the frequency of the incident radiation provided the frequency exceeds a certain limit.*

Without this tremendous display of energy, by merely putting a substance in solution electrons are freed. The necessary energy might come from all or any of the heat liberating actions that take place during the process of solution (*e.g.*, heats of hydration and dilution, heat of combination of the anion with an extra valence electron), and the process might be aided by the heat of hydration of the ions as they are set free. The whole subject is bristling with difficulties, and so far explanations can only be regarded as reasoned guesses.

Water.

The solutions dealt with above have all been aqueous. Solutions with water as the solvent were early recognised as the most important. According to the old Greek philosophers water was " the beginning of all things." Thales said, " All things have their origin in water and return unto the same." Aqueous solutions are fundamental for all biological phenomena. The physical properties of water are in general *extreme*—their numerical expressions are either extremely large or extremely small, and usually the former. Its specific heat and its dielectric constant are the highest of any of the more common liquids. Therefore, water should have a very high ionising power as a solvent.

One has been accustomed to look upon water as a simple inert substance, of the chemical formula H . OH and with a molecular weight of 18. Physical chemists have proved that this conception does not account for all the properties of water. Lewis and also Langmuir, from thermodynamical principles and also from the study of the colligative properties (see p. 41) of water, have constructed diagrams of the molecule of water. Discussion of this work is somewhat without the bounds of this book.

In recent years it has been amply demonstrated that a triatomic molecule could not possess the properties of water. For instance,

it is composed of gases with extremely low freezing and boiling points. Oxygen boils at $-181°$, while the figure for hydrogen is $-253°$ C. From comparison with compounds of known composition, ice should form at $-150°$ and the temperature of steam should be $-100°$ C. The inference from this is that the molecule of water is bigger than H_2O. Each simple molecule or hydrol is supposed to combine with another hydrol so as to form a dihydrol, or three hydrols may polymerise to trihydrol, and so on. Water, as we know it, consists of a mixture of these various hydrols. The relative amount of each kind is determined (*a*) by the temperature of the fluid, and (*b*) by the substances present in solution or, in a less degree, in suspension.

(*a*) **Temperature** controls the kinetic energy of the molecules, and so the size of the intra-molecular spaces. Increase of temperature, therefore, by increasing the kinetic energy will cause a disruption of polyhydrol into its simpler constituents. Decrease of temperature has the reverse effect. Theoretically, there is the gas H_2O and the solid $(H_2O)_3$, and between these extremes the liquid $(a(H_2O) + b(H_2O)_2 + c(H_2O)_3)$, a, b and c being constants dependent on the temperature. At each temperature there is equilibrium between the amounts of the various hydrols. The temperature of water has thus an importance in deciding its physical and chemical properties, and therefore, *in all reactions involving water, temperature should be stated.*

(*b*) As has been pointed out above, there is a certain equilibrium composition of water at each temperature. This equilibrium is disturbed by the presence of a solute, especially if it is dissociated. Hydrol is abstracted to hydrate the ions or molecules of the solute and a rearrangement of equilibrium takes place.

Ionisation Constant.

Absolutely pure water is almost, but not quite, a non-electrolyte. As absolutely pure water has not yet been prepared, this is a deduction from the behaviour of water under certain circumstances.

Water is ionised according to the equation

$$H_2O \rightleftharpoons H^+ + OH^-.$$

According to Guldberg and Waage's Law of Mass Action, the product of the concentrations of the reacting substances, H^+ and OH^-, bears a direct relationship to the mass of the resultant substance H_2O.

That is $$\frac{[H^+] \times [OH]^-}{[H_2O]} = \text{constant } K,$$

where $[H^+]$ = the molecular concentration of hydrogen ions.
$[OH^-]$ = the molecular concentration of the hydroxyl ions.
and $[H_2O]$ = the molecular concentration of the undissociated water.

In practice it is found that so little water is dissociated that relative to [H] and [OH], $[H_2O]$ is constant. $K[H_2O]$ is thus constant and equal to K_w, which is defined as the dissociation constant of water.

The value of K_w, the dissociation constant of water, depends only on the temperature.

At 0° C. $$K_w = \frac{1}{10{,}000{,}000{,}000{,}000{,}000} \quad . \quad . \quad . \quad . \quad (1)$$

At 22° C. $$K_w = \frac{1}{100{,}000{,}000{,}000{,}000} \quad . \quad . \quad . \quad . \quad . \quad (2)$$

At 40° C. $$K_w = \frac{3{\cdot}5}{100{,}000{,}000{,}000{,}000} \quad . \quad . \quad . \quad . \quad . \quad (3)$$

At 100° C. $$K_w = \frac{48}{100{,}000{,}000{,}000{,}000} \quad . \quad . \quad . \quad . \quad . \quad (4)$$

To save writing those cumbrous fractions, the index notation is used. Thus fraction

(1) is $= 0{\cdot}01 \times 10^{-14} = K_w$ at 0° C.
(2) is $= 1 \times 10^{-14} = K_w$ at 22° C.
(3) is $= 3{\cdot}5 \times 10^{-14} = K_w$ at 40° C.
(4) is $= 48 \times 10^{-14} = K_w$ at 100° C.

Since $$[H^+] \times [OH^-] = K_w,$$
and obviously H^+ and OH^- are produced in equal amounts,
therefore $$[H^+] = [OH^-] = \sqrt{K_w}.$$

Between 22° and 23° C. water has a dissociation constant with which it is convenient to work, and measurements of hydrogen ion concentrations are usually made at this temperature or referred to this temperature;

$$i.e. \text{ at } 23° \text{ C., } K_w = 10^{-14};$$
$$\therefore [H^+] \times [OH^-] = 10^{-14}.$$
$[H^+]$ is therefore equal to $\sqrt{10^{-14}} = 10^{-7}$,
and $[OH^-]$ „ „ „ $\sqrt{10^{-14}} = 10^{-7}$.

It is usual to write H· for H^+ and OH′ for OH^-.

Still further to shorten the symbols, Sørensen suggested the use of the logarithm to denote the hydrogen ion concentration.

Instead of writing 10^{-7} one may write merely the positive index 7, keeping the rest of the formula in mind. This is called the p_H, p denoting the index to the base 10, and H, of course, showing that hydrogen ions are under consideration. That is, in neutral water at about 23° C.

$$p_H = p_{OH} = 7,$$

or

$$C_H = C_{OH} = 10^{-7}.$$

In words, neutral water has a **hydrogen ion concentration** of 10^{-7} or a p_H of 7.

Appended is a list of values of p_H of water for various temperatures.

TABLE VIII

EFFECT OF ALTERATION OF TEMPERATURE ON THE DISSOCIATION OF WATER

Temperature.	pH.	pOH.	pH_2O.
16° C.	7·10	7·10	14·2
18° C.	7·07	7·07	14·14
20° C.	7·03	7·03	14·06
22° C.	7·0	7·0	14·0
24° C.	6·96	6·96	13·92
26° C.	6·93	6·93	13·86
28° C.	6·90	6·90	13·80
37° C. (body temp.) . .	6·75	6·75	13·5

Some people prefer a more cumbrous but nevertheless a more comprehensible method of recording ionic concentrations. In Sørensen's method it is rather difficult to see at a glance the relative concentrations of ions at two temperatures. As the temperature increases, dissociation increases, but the negative exponent or p_{H_2O} decreases. At 8°, for example, the p_H is 7·3, and at 22° it is 7·0. Put in this way, one does not readily grasp the fact that the p_H at 22° is double the p_H at 8°. If, however, the negative exponent be kept a whole number and the fraction be put as a *multiplier*, the relation is seen at once, *e.g.*,

$$8° \; p_H = 7{\cdot}3 = 0{\cdot}5 \times 10^{-7} = C_H,$$

$$22° \; p_H = 7{\cdot}0 = 1 \quad \times 10^{-7} = C_H.$$

The conversion of one expression into the other is simple. For example : To convert p_H 7·6 to other notation

$$p_H \; 7{\cdot}6 = 10^{-7{\cdot}6} = 10^{-7{\cdot}0} \times 10^{-0{\cdot}6} = 0{\cdot}25 \times 10^{-7}$$

$$\text{antilog of} - 0{\cdot}6 = 0{\cdot}25.$$

Conversely we find the short expression for

$$\log C_H\, 5 \times 10^{-6} = \log 5 + \log 10^{-6}$$
$$= \cdot 6990 + (-6\cdot 0000)$$
$$= -5\cdot 3 \text{ i.e. } p_H, = 5\cdot 3$$

or $$C_H\, 5 \times 10^{-6} = 10^{\cdot 699} \times 10^{-6} = 10^{-5\cdot 3} \equiv p_H\, 5\cdot 3.$$

Graph for conversion from one notation to other, Part II., p. 564.)

Reaction to Indicators.

It is very important to be able to ascertain with great exactness, the true acidity or alkalinity of physiological media. It is not sufficient to state that a certain fluid is acid to litmus, etc. Litmus, for one thing, is not nearly sensitive enough to indicate the minute changes in reaction which alone are of physiological value. The whole activity, of the mammal, at any rate, is regulated by reaction. Alterations in acidity are the causative factor in the regulation of respiration, the activity of muscle, the excitability of nerve, and play an important part in regulating secretion and excretion. Physical and chemical means are employed to keep the healthy body within a narrow range of reaction, about the neutral point. Any marked deviation from this is pathological, and is the result of pathological (or experimental) conditions. As we shall see later, the neutrality of the organism is an equilibrium, any disturbance of which will produce change, and, moreover, any change in the organism will tend to disturb this equilibrium (Chap. XXXI.).

Examination of a number of acids shows that when they dissociate in water they disturb the balance existing between the concentrations of H^+ and OH^- ions.

For example :

$$HCl = \mathbf{H}^+ + Cl^-$$
$$HNO_3 = \mathbf{H}^+ + NO_3^-$$
$$H_2SO_4 = \mathbf{H}^+ + HSO_4^-$$
$$CH_3COOH = \mathbf{H}^+ + CH_3COO^-$$

In each case the acid produces $\mathbf{H}^+$ ions. Now, as $[H] \times [OH]$ is a constant, the result of this increase of H^+ ions must cause a decrease in the concentrations of OH^- ions.

In the same way, examination of the behaviour of alkalies shows also a disturbance of the ratio of $[H^+]$ to $[OH^-]$.

For example :

$$NaOH = Na^+ + \mathbf{OH}^-$$
$$NH_4OH = NH_4^+ + \mathbf{OH}^-.$$

The concentration of OH^- ions is increased.

In water the concentrations of H and OH are equal. These facts lead to the following definitions :

(*a*) Any substance which when dissolved in water yields $\mathbf{H}^+$ as one of the direct products of its ionisation is an **acid.**

(*b*) Any substance which when dissolved in water yields $\mathbf{OH}^-$ as one of the direct products of its ionisation is a **base.**

(*c*) Any substance which on ionisation yields at least one positive ion other than H^+ and at least one negative ion other than OH^- is a **salt.**

(*d*) If, in addition to the positive and negative ions mentioned in (*c*), the salt yields an H^+ ion, it is called an **acid salt.**

E.g.

$$KHSO_4 = K^+ + SO_4^{--} + \mathbf{H}^+$$
$$Na_2HPO_4 = 2Na^+ + PO_4^{---} + \mathbf{H}^+$$
$$\begin{array}{l} COOH \\ | \\ COONH_4 \end{array} = NH_4^+ + (COO)_2^{--} + \mathbf{H}^+$$

(*e*) If, in addition to the positive and negative ions mentioned in (*c*), the salt yields an **OH**-ion, it is called a **basic salt.**

E.g.

$$Fe(OH)_2Cl = Fe^{+++} + Cl^- + 2\mathbf{OH}^-,$$
$$CH_2 \cdot NH \cdot OH \cdot COOK = K^+ + CH_2NHCOO + \mathbf{OH}^-.$$

(*f*) Substances which produce both H· and OH′ ions on dissociation are called **amphoteric** electrolytes or ampholytes. They must evidently have two ionisation constants, K_H and K_{OH}. It is obvious that acidity depends on the preponderance of hydrogen ions over hydroxyl ions, and conversely, alkalinity is due to the presence of hydroxyl ions in excess of hydrogen ion Neutrality is an equilibrium between H· and OH′.

This neutral point occurs in water at 23° C. when th tion of hydrogen ions is 1×10^{-7}, *i.e.* $p_H = 7$. If the co is greater than this, *e.g.* 1×10^{-5}, or $p_H = 5$, then the tion of OH′ must be correspondingly decreased accord equation,

$$[H^+]\,[OH^-] = 10^{-14}$$

or
$$[OH^-] = \frac{10^{-14}}{(H^+)} = 10^{-14\,(-5)} = 10^{-9}.$$

A p_H of 5 will be accompanied by a p_{OH} of 9. This will acid solution.

Conversely, if the concentration of hydroxyl ions is inc ased, there is a corresponding decrease in hydrogen ions. *E.g.*

$$\text{if } p_{OH} = 3, \text{ then } (H^+) = \frac{10^{-14}}{10^{-3}} = 10^{-11} = p_H \text{ of } 11,$$

an alkaline solution.

Reaction may, therefore, be expressed in terms of p_H or of p_{OH}. Generally the former is used, and alkalinity is expressed as decrease of acidity. The quality as well as the nature of the reaction is expressed by the p_H. The greater the concentration of hydrogen ions, the greater is the degree of acidity and the smaller the degree of alkalinity.

It is rather confusing for the beginner, but he must note :

(1) that as acidity increases, the exponent or *p figure* decreases ;

(2) that as the *figures* are logarithms, multiplication is done by addition and division by subtraction.

(3) that this does not give a measure of the *amount* of acid present, but of its *strength*. The p_H is not an index of quantity but of intensity. It gives the number of H ions per litre, but of course says nothing of how many litres or c.c. of acid are present.

The concentration of an acid (or alkali) may be expressed as **normal** or as a fraction of normal. A normal solution contains in one litre, the gram-equivalent weight of the substance. A normal solution of acid, for instance, has in each litre one gram of hydrogen capable of forming hydrogen ions. If the acid is completely dissociated, *i.e.* if it is a " strong " acid, it will contain one gram of hydrogen as H^+. The concentration of acid commonly used for laboratory purposes is 1/10 of normal $= \frac{N}{10}$. The hydrogen ion concentration of such a solution would be 1/10 gram per litre $=$ (H^+) of 1×10^{-1} or p_H of 1 and p_{OH} of 13.

Water of $p_H = p_{OH} = 7$ is thus, at 23° C., N/10,000,000 acid and N/10,000,000 alkaline. If the acid added to water is not completely dissociated (*i.e.* a weak acid), then, of course, the degree of dissociation must be taken into account. A decinormal solution of acetic acid, for instance, at 23° is dissociated 1·36 per cent. Therefore its (H^+) would be equal to $\frac{1{\cdot}36}{100} \times 10^{-1} = 1{\cdot}36 \times 10^{-3}$ or p_H of 2·86.

Normal solutions of acid are all equal as regards the amount of alkali they can neutralise. 1 c.c. of any N/10 acid is exactly neutralised by 1 c.c. of any N/10 alkali. That is, they have the same titratable acidity. They differ in their concentration of hydrogen ions.

As we have seen

$$\frac{N}{10}\ \text{HCl in water at 23°} = p_H\ 1 \text{ or } C_H = 1 \times 10^{-1},$$

$$\frac{N}{10}\ CH_3COOH \quad \text{,,} \quad = p_H\ 2{\cdot}86 \text{ or } C_H = 1{\cdot}36 \times 10^{-3}.$$

That is, hydrochloric acid, under the above conditions, has

$$10^{-1} \div 10^{-2.86} = 73.5$$

times the amount of hydrogen ions per litre that acetic acid has. N/10 Hydrochloric acid at 23° C. is therefore 73·5 times as strong as N/10 acetic acid.

Salts.

It is very seldom that acids, weak or strong, occur alone or diluted with water in physiological fluids. Salts are always present. In (*d*) and (*e*) are mentioned two classes of salts which alter the [H] of water when dissolved in it. They do so *directly* in virtue of their possession of an additional H· or OH′ ion.

Other salts cause alterations in acidity by upsetting the balance between H· and OH′ in water. Their action is *indirect.*

(1) The salt of a strong acid and a strong base, *e.g.* NaCl, causes little or no change in [H].

(2) If one of the constituents of a salt be weak, changes occur.

(*a*) If the salt BA of the strong base B . OH and the weak acid HA be dissolved in water, it forms $BA = B^+ + A^-$. But owing to the ionisation of the solvent there are present H^+ and OH^- ions and a second change takes place, for H^+ and A^- ions are present. According to the law of mass action

$$\frac{[H\cdot] \times [A']}{[HA]} = K_{HA} \text{ or } H\cdot + A' \rightleftharpoons HA.$$

As no HA is present to balance the reaction, H· will combine with A′ to form HA until the point of equilibrium for that dilution has been reached.

Summarising these reactions as follows :

$$A' + H_2O = HA + \mathbf{OH}',$$

the net result is the liberation of OH ions. *The addition of a salt of a strong base and a weak acid is to make the solution alkaline, i.e.* to reduce the hydrogen ion concentration. This is a fact of great physiological importance, as most of the salts of the body are composed of organic acids combined with the strong bases sodium and potassium.

KCN, a powerful poison, dissociates as follows :

$$\left.\begin{array}{l} KCN = K^+ + CN^- \\ H_2O \ = H^+ + OH^- \end{array}\right\} = HCN + K^+ + \mathbf{OH}^-.$$

This causes an alkalinity equal to that of potassium hydrate. The alkalinity of solutions of sodium carbonate is due to the reactions,

$$
\begin{array}{lll}
Na_2CO_3 & = 2Na^+ & + CO_3^- \\
H_2O & = H^+ & + \mathbf{OH^-} \\
CO_3^- + H_2O & = HCO_3^- & + \mathbf{OH^-} \\
HCO_3^- + H_2O & = H_2CO_3 & + \mathbf{OH^-} \\
H_2CO_3 & = H_2O & + CO_2
\end{array}
$$

If the CO_2 is allowed to escape, the last reaction will only cease when all the H_2CO_3 has been decomposed. The total result is an increase in [OH] and, therefore, of alkalinity.

(*b*) In the case of a weak base combined with a strong acid, the solutions become acid, as the following equations denote.

$$
\left.
\begin{array}{llll}
BA = & B^+ & + & A^- \\
H_2O = & H^+ & + & OH^-
\end{array}
\right\} = BOH + \mathbf{H^+} + \mathbf{A^-}
$$

where HA is a strong acid and BOH a weak base.

$$
\textit{E.g.} \qquad
\left.
\begin{array}{llll}
NH_4Cl = & NH_4^+ & + & Cl^- \\
H_2O = & H^+ & + & OH^-
\end{array}
\right\} = NH_4OH + \mathbf{H^+} + \mathbf{Cl^-}.
$$

(3) When both the constituents are weak the solution will remain neutral, if acid and base are of equal strength; if the acid be the stronger, the solution will be acid, and conversely an alkaline solution will be produced if the base be stronger than the acid. *E.g.*

$$CH_3COONH_4 + H_2O = NH_4OH + CH_3COOH.$$

This solution will be almost neutral, because the degrees of ionisation of ammonium hydrate and acetic acid are almost identical.

Effect of Temperature.

The effect of temperature on the dissociation of water has been dealt with above (p. 62 and Table VIII.). Increase in temperature causes a very large increase in the amount of water ionised. An increase in temperature of 1° C, say from 37° to 38°, causes the [H] × [OH] to rise from $10^{-13.5}$ to $10^{-13.47}$, an increase of about 10 per cent. Strong electrolytes have a low temperature coefficient of dissociation. It is, therefore, obvious that increase of

temperature will affect salts according to the dissociation constant of the acids and bases composing them.

(*a*) Both strong, temperature of little effect.

(*b*) Weak acid + strong base. Increase of temperature causes the degree of dissociation of acid to increase. Anions combine with hydrogen ions from H_2O and liberate $\mathbf{OH}^-$.

(*c*) Strong acid and weak base. Increase of temperature causes the degree of dissociation of base to increase. Base ions combine with hydroxyl ions from the H_2O and liberate $\mathbf{H}^+$.

(*d*) Both weak. The result of any increase in temperature is to increase the dissociation of the weaker at a greater rate than the stronger with correspondingly slight changes in [H] and [OH].

It will be seen that apart from the action of temperature on the dissociation of water itself, in (*b*) increased alkalinity and in (*c*) increased acidity result from increase in temperature. This action is slight, however, compared to the action of temperature on the weakest salt known, water.

The effect of alterations of temperature on a salt solution where one of the constituents of the salt is weak is the combined effect of

I. the alteration in K_{H_2O};
II. the alteration in K_{Salt}.

In brief, the increased acidity or alkalinity produced by increase of temperature is greater (theoretically) than could have been produced from increased dissociation of the salt. The significance of this will be seen later.

At present the point under consideration is the mechanism for converting the potential energy of the food-stuffs into the kinetic energy exhibited by protoplasm. Enough has been said to indicate

(1) That slight alterations in hydrogen ion concentration may produce large alterations in surface tension (Chap. VI.).

(2) That slight alterations in hydrogen ion concentration may produce large alterations in the degree of dissociation of salts.

(3) That the degree of dissociation of salts, acids and bases governs the value of surface tension and osmotic pressure. The next chapter deals with the inactivation of these factors.

Further Reading

Crocker & Matthews. "Theoretical and Experimental Physical Chemistry." J. & A. Churchill.

CHAPTER VIII

DISPERSE SYSTEMS

I. COLLOIDS—THE RESERVOIRS OF ENERGY

"The properties of colloidal solutions can be most efficiently inquired into by application, as far as possible, of the same views and methods as those generally applied to true solutions." SØRENSEN.

PROTOPLASM consists largely of water. For instance, about 85 per cent. of the total body weight of a puppy is water. This water is partly "free," *i.e.* may readily be removed by gentle drying, and partly "bound," removable only by destruction of the tissues. The bound water may amount to as much as 1·8 grams for every gram of dry matter in the animal. Obviously, some mechanism must exist to keep this fluid in position and so to mask it as to give the impression of more or less solid tissue. The part of water-holders is played by colloids, emulsions and certain crystalloids.

In Chap. V. colloids were mentioned as a series of substances which when dissolved in water have a lower osmotic pressure than would be expected from their molecular weight. The reason for this, deduced from the colligative properties of their solutions, is that in water they form aggregates or particles of extra-molecular size.

The effect of this is enormously to increase the effective surface of the solvent. Therefore the phenomena of surface tension and surface adsorption will be marked.

The appended table makes clear the enormity of the increase in surface that takes place when a sphere is divided into a large number of small shot and these are, in turn, divided into particles of colloidal size (Table IX.).

This table shows how a molecular solution of particles of 0·1 $\mu\mu$ radius acquires an additional effective surface of 12,600 sq. metres when the particles are increased in size sufficiently to bring them into the colloidal realm. A surface is *effective* when its area is large enough to accommodate the heads (or tails as may be) of molecules which may be held end-on to it. The diameter of the cross-section of most molecules can readily be calculated, and so the possibility of their adsorption to particles of any particular size may be predicted. In this connection Wo. Ostwald has

TABLE IX

INCREASE IN SURFACE OF A SPHERE WHEN ITS RADIUS IS DECIMALLY DIVIDED

Length of Radius.		Number of Spheres.	Total Surface.
1 cm.	Small Shot	1	12·6 sq. cm.
1 mm.		10^3	126 ,,
0·1 mm.	Coarse	10^6	1260 ,,
0·01 mm.	Suspensions	10^9	1·26 sq. metres
1μ		10^{12}	12·6 ,,
$0{\cdot}1\mu$	Typical	10^{15}	126 ,,
$0{\cdot}01\mu$	Colloids	10^{18}	1,260 ,,
$1\mu\mu$		10^{21}	12,600 ,,
$0{\cdot}1\mu\mu$	True Solution	10^{24}	126,000 ,,

introduced the term *specific surface* to denote the ratio of surface to volume or S/V. In a sphere $S = 4\pi r^2$ and $V = \frac{4}{3}\pi r^3$: therefore $\frac{S}{V} = \frac{4\pi r^2 \times 3}{4\pi r^3} = \frac{3}{r}$. It has been found in physical chemistry that adsorption to a surface becomes an important factor when the *specific surface* reaches a value of about 10,000. It has also been noticed that when the specific surface becomes greater than 6×10^7 approx., *i.e.* when the material is so finely subdivided that it is in molecular solution, adsorption phenomena cannot be detected.

Crystalloids and Colloids. Protoplasm, composed of proteins, lipides, carbohydrates, organic and inorganic salts and a large amount of water, is enclosed within a plasma-membrane, which permits of the free passage of water, certain salts and other substances, but not of protein and similar complexes. If a mixture of, say, albumin, starch, glucose, common salt and water were enclosed in a parchment bag suspended in water, the glucose and salt would pass through the membrane into the external water, while the albumin and starch would remain within the bag. Substances which pass readily through membranes like parchment are termed *crystalloids*, and the albumin-like substances *colloids*.

The division is due to Graham, the pioneer in colloidal research. As the result of a large series of investigations on the rates of diffusion of various substances in water, he was led to divide all substances into two classes, *e.g.* crystalloids, which have a high rate of diffusion and which crystallise from saturated solutions, and colloids, which diffuse very slowly and in general have a gluey consistency. " They appear," he writes, " like different worlds of

matter, and give occasion to a corresponding division of chemical science." The process of separating crystalloids from colloids by means of a membrane is called *dialysis*. (See Part II., Experiment 26.)

It has now been proved that matter may exist either in a crystalloidal or in a colloidal state, and that by suitable means a colloid may be crystallised and so pass through a membrane previously impermeable to it. The converse process may also take place. The solvent is sometimes the factor on which depends the state of the solute. The alkali salts of the higher fatty acids—stearic, palmitic, oleic—form a true molecular solution in alcohol, but with water they act as colloids. On the other hand, sodium chloride, a typical water-soluble crystalloid, assumes the colloidal state in benzol. Von Weimarn and others have prepared colloidal solutions of over two hundred substances usually considered as crystalloids. By proper manipulation, almost any solid can be dispersed through a liquid either as a crystalloid or as a colloid. Consequently, one now speaks of the **colloidal state** rather than of certain substances as being colloids.

The difference between a crystalloidal and a colloidal solution depends, in the main, on the size of the particle in the fluid.

There is some difficulty in expressing the relationship between the colloid and the fluid in which it is. It is not in true solution, but is suspended and dispersed throughout the medium. The colloid may, therefore, be called the dispersed substance or dispersate and the fluid the dispersing medium or dispersant.

The application of the " Phase Rule " (of W. Gibbs) has helped to clear up several difficulties in physiological physics, and some writers have adopted terminology suitable for use when this rule is discussed. It is sufficient here to say that the dispersed phase is the substance which is suspended or distributed throughout the continuous phase. As an illustration, attention may be drawn to a disperse system having two phases and only one component, *e.g.*, a fine mist of liquid water suspended in water vapour. The dispersed, internal or non-continuous phase is composed of the droplets of water ; the continuous or external phase, or dispersion medium, is the water vapour. The stability of this dispersion depends on two factors, (*a*) the temperature of and (*b*) the diameter of the droplets. (Such a system is called *divariant.*) The smaller the droplets, the greater is the ratio of surface to mass and the higher is the vapour pressure. All the droplets will not be of the same size, and therefore the larger droplets will tend to become larger still at the expense of the smaller ones. The system is, on this account, said to be *metastable.*

Disperse systems may be classified according to *the nature of the contact surface* between the phases. Taking the three states of matter, solid, liquid and gaseous, five different kinds of contact surface can be produced, as is indicated in the following table, in which are also given examples of the various disperse systems.

TABLE X

Class.	Contact Surface.	Dispersed Phase.	Continuous Phase.	Examples.
I.	Gas—Liquid	Gas Liquid	Liquid Gas	Foam, Suds, Lather. Mist, Spray, Steam.
II.	Gas—Solid	Gas Solid	Solid Gas	Hydrogen in platinum; lava, meringues, meerschaum. Smoke, dust and some fumes.
III.	Liquid—Liquid	Liquid	Liquid	Milk (fat in water), Lymph, egg white (raw).
IV.	Liquid—Solid	Liquid Solid	Solid Liquid	Rock inclusions. Opal. Colloidal metals, etc.
V.	Solid—Solid	Solid	Solid	Ruby Glass (Gold in Glass). Some precious stones.

Class III. (So-called Emulsoid) is of the most importance in biology, but Class IV. (Suspensoid) has been most studied and is of considerable industrial and therapeutic value.

Colloids might be classified according to their **degree of dispersion**, that is the ratio of total surface to volume, or the surface exposed to each c.c. of the dispersed phase. This would give a continuous series of systems ranging from a non-dispersed two-phase system on the one hand to a homogeneous mixture of an ionised salt in water, *i.e.* a true solution. Colloids may thus be regarded as intermediate in this series, *e.g.*, gold coin in water, gold dust suspended in water, very fine gold dust suspended in water, range of colloidal gold in water (Zsigmondy), solution of gold salt (undissociated) and, finally, completely dissociated gold salt in aqueous solution.

This state is not peculiar to the metallic colloids, but, as has been amply demonstrated by Von Weimarn, can be obtained from such materials as NaCl, $Al(OH)_3$ and silver salts. He has enunciated a postulate called **the law of corresponding states**, which is as follows: "The degree of dispersion and the general physical appearance of precipitates are always the same irrespec-

tive of the chemical nature of the precipitates provided that the precipitation takes place under *corresponding conditions.*" Working with substances as widely apart in their chemical nature as the various salts of aluminium, barium, silver, sodium and many others, Von Weimarn has prepared precipitates with almost any desired degree of dispersion ranging *in each instance*, all the way from coarse and obviously crystalline precipitates, to gelatinous precipitates and thick transparent jellies.

Physiological colloids differ from this metallic series in one respect at least. They dissolve in water and they also imbibe water. A solution of albumin, for instance, cannot be regarded as a solid dispersed throughout a liquid, but is a strong solution of albumin dispersed throughout a weaker solution. Because of their affinity for water such colloids are termed hydrophilic, *i.e.* water-loving, in contradistinction to the hydrophobic suspensoids, which are readily separated from their dispersion medium.

Colloidal matter may be further divided into two groups. White of egg is a hydrophilic colloid. In its ordinary state, as obtained from the egg, it can be dissolved in water to form a clear solution. Boiling the solution causes coagulation of the egg white. It comes out of solution in the form of a white semi-solid, insoluble in water. Those colloids which form solutions like egg white are called **sols**. According to the medium in which they were dispersed they were termed by Graham, hydrosols, alcosols, glycerosols, etc. Colloids which assume a semi-solid form like coagulated egg white are called **gels**. In a gel the more liquid phase is dispersed through the less liquid phase (see p. 98).

Preparation of Colloidal Dispersions.

Some substances easily assume the colloidal state, but fairly strenuous methods have to be adopted to induce others to do so. The naturally occurring colloids, such as proteins of all kinds and polysaccharides, are caused to crystallise with difficulty, while substances which crystallise easily become colloids under compulsion. The methods used in the preparation of colloids fall, in general, into two classes, chemical and electrical methods. In the former class is included all methods which entail reduction, double-decomposition, hydration, substitution of solvent, peptisation, etc. In Part II. are given directions for the preparation of typical colloids by these methods. Electrical dispersion methods consist in the passage of an oscillating discharge between iron or aluminium electrodes immersed in water (or other dispersion medium) in which are suspended coarse fragments of the metal to be dispersed.

Properties of a Colloidal Dispersion.

The properties of a dispersion depend in general either on the *size* of the dispersed particles or on their *electrical charge*, or on both.

1. PROPERTIES OF COLLOIDS DEPENDING ON SIZE OF PARTICLES

(i) Optical.

(*a*) **Colour.** White light is composed of waves of different lengths varying from $760\mu\mu$ to $450\mu\mu$. When white light is scattered from a surface instead of being reflected as in a mirror, it gives rise to the sensation of white. Ice, in mass, does not appear white because light is not scattered from its surface. If the ice is powdered, light is scattered from the powdered surfaces and the whole appears white. Crystallised copper sulphate appears blue, but the light scattered from the surfaces of the finely powdered crystals is white. The white colour of the lily or of white hair is not due to the presence of a white pigment, but to the scattering of light from the surfaces of innumerable minute air bubbles embedded in the tissue. From this it follows that particles of different sizes will scatter light of different wave-lengths. In short, the colour of the scattered light may serve as an indication of the size of the particle, provided the difference in the indices of refraction of the dispersoid and the dispersant be kept constant.

The late Lord Rayleigh deduced a formula relating the size of the particle and the wave-length of the scattered light in a quantitative manner. A particle smaller in diameter than half the wave-length of light will scatter light at the blue end of the spectrum about twelve times as copiously as it does the longer red rays.

He explained the blue colour of the sky by considering that the fine particles of dust, globules of water, etc., suspended in the air, or even the molecules of the various gases of the atmosphere, cause lateral diffusion of light of short wave-length giving a blue colour, while the red rays are transmitted direct, producing the gorgeous sunset colours. In one of Tyndall's experimental verifications of this theory he passed light through a tube containing a mixture of gases (butyl nitrate in air and hydrochloric acid in air), which gradually combined to form a dust-like suspended precipitate. At first the particles were exceedingly small and the colour seen from the side of the tube was a delicate tint of blue. As the particles increased in size the blue became more intense, " until at length a whitish tinge mingled with the pure azure, announcing that the particles were now no longer of that infinitesimal size which scatters only the shortest waves."

The colour of some samples of stained glass is caused not by an even distribution of the pigment or stain throughout the glass, but by the dispersion of fine metallic particles. Water of sufficient depth appears blue because of the presence of tiny suspended particles. If larger particles are present, some light of longer wave-length, *e.g.* yellow, is diffracted and the colour becomes green. The water of the Rhone as it leaves Lake Geneva is intensely blue, while the Rhine at Strassburg is green. The Rhine contains about 70 per cent. more calcium carbonate in suspension than the Rhone.

Tyndall observed that the blue of the eye has a similar origin to the blue of the sky, the sea, and the Rhone, *viz.* scattering of light from small suspended particles. The uvea, the dark pigmented double layer at the back of the iris, prevents the reflection of light and prevents the colour of the blood in the vessels behind it from becoming apparent. In an albino this pigment is absent and the eye appears pink. The colour of blue eyes is due to fine unpigmented colloid particles suspended in the iris. The various colour stages between the blue and the grey eye arise from differences in the mean size of the dispersoid particles—the finer the particles, the more intense the blue. In brown and black eyes, pigment cells are found in the endothelium in front of the iris. Except with people who have very black eyes, the pigment on the anterior surface of the iris does not develop at birth. That is, most babies are born with deep blue eyes. As they become older the colloidal particles become larger and the blue becomes less intense. Further, if the pigment develops the colour changes from blue to hazel, brown or black. The reverse change never takes place (Bancroft).

Colour may be due, as we saw in Chap. II., to the reflection of non-absorbed light. A surface which completely absorbed light would give rise to the sensation of black, while a perfect reflecting surface would be, of course, invisible. It follows that particles of different sizes will " select " light of certain wave-length for absorption, and, as a consequence, colour may result from " selective " absorption, reflection or diffraction.

In the table on p. 78, from Ostwald, is given the relationship between size of particle and colour (*a*) from light absorbed, and (*b*) from light transmitted (Table XI.).

One must, however, take into account the other optical components, *e.g.*, refractive index of medium. The absorbed colour given below does not necessarily indicate the colour of light scattered by the particles.

As the particle becomes smaller, the colour transmitted alters to

TABLE XI

CORRESPONDING ABSORBED AND SUBJECTIVE COLOURS

(*a*) Wave-length in μ	·70	·65	·60	·55	·53
Absorbed Colour	Purple	Red	Orange	Yellow	Green-Yellow
(*b*) Transmitted Colour	Green	Green-Blue	Blue	Indigo	Violet
Wave-length in μ	·50	·48	·45	·43	·40
(*a*) Wave-length in μ	·50	·48	·45	·43	·40
Absorbed Colour	Green	Green-Blue	Blue	Indigo	Violet
(*b*) Transmitted Colour	Purple	Red	Orange	Yellow	Green-Yellow
Wave-length in μ	·70	·65	·60	·55	·53

light of longer wave-length, *e.g.* from blue or green, through various shades of yellow and orange to red. If the suspended particles are very fine, blue light is, as we have noted above, scattered laterally, while red light is transmitted. Such a system will appear red by transmitted light and blue by reflected light (*e.g.* skim-milk, tobacco smoke and colloidal gold). It has also been shown that as the particles decrease in size, the absorption bands in the spectrum of the solution shift towards the ultraviolet (Ostwald).

OPTICAL RESONANCE.—The amplitude of vibration of a particle is a function of its mass, temperature being kept constant. As the mass alters so will the period of vibration. According to Wood, metallic particles, if highly dispersed, owe their colour not to ordinary reflection, diffraction, interference, etc., but to optical resonance. Resonance is the production of vibrations in a body by the periodic application of a stimulus which has the same period as the natural period of the body. The vibrations of a tuning fork may be transmitted through the air and cause to vibrate another tuning fork of the same pitch. Since the resonator owes the energy necessary to set it into vibration to the stimulating body, it follows that the stimulating body must lose energy to the resonator. The particles in colloidal solution are supposed to be vibrating with the same frequency as light of a certain wave-length. Consequently, they will receive energy from the light which will tend to increase their amplitude of vibration. The kinetic energy of the solution will tend to increase, but any increase in kinetic energy would mean increase in temperature and a slight alteration

in frequency. This opens up the possibility of considerable energy changes in comparatively short times.

What effect will be produced when the rates of vibration are nearly but not quite the same ? If two pendulum-controlled clocks which are keeping nearly the same time when on separate stands are placed on the same stand they will keep time exactly. Both pendulums transmit vibrations to the stand, and so to one another. The faster pendulum exerts a periodic force on the slower pendulum and is itself slowed by the loss of energy. In the same way the slower pendulum tends to cause forced vibrations in the stand and so influence the faster pendulum. Finally the two pendulums (and stand) vibrate at periods exactly the same. Is it possible that light may cause forced vibrations of colloidal particles ?

Certain investigators have claimed that the Brownian movement may attain an increased velocity because of incident light. Exner found that exposure to light of a suitable wave-length had a slight but a positive accelerating effect.

One effect of optical resonance is the production of surface colours. When light of a certain wave-length is strongly absorbed by particles, they may also reflect that light " selectively." For instance, magenta crystals (aniline dye) transmit red but reflect green. If the particle is made small enough it will scatter the light that it previously transmitted, and will transmit, of course, the light that is not scattered. This is readily carried out with indigo. In mass, *i.e.* when the particles are large, this colloidal dye appears red when observed laterally to the plane of incidence of light. By transmitted light it is blue, *i.e.* appears blue when looked at against the light. If a fine suspension is prepared it reflects blue and transmits red.

(*b*) **Faraday-Tyndall Phenomenon.** An examination of the optical properties of these various disperse systems makes it clear that there is a regular gradation in the size of the particles dispersed, which passes from the easily visible suspension to the invisible solute. If the size of a particle is decreased below $200\mu\mu$, it cannot be seen even by the most powerful microscope made, or that could ever be made. The particle is ultra-microscopic because its diameter is less than half the wave-length of light. If monochromatic light with a very short wave-length, say 2,000 Å.u., were to be used with a suitable microscope, particles of $100\mu\mu$ and greater could be *photographed.* Such a microscope with quartz lenses has been constructed by Barnard.

The tiny particles may also be made apparent in much the same way as the innumerable specks of dust floating in the air become sparkling motes dancing in a ray of sunlight which has penetrated into a partially darkened room. When a strong beam of light is sent through a rectangular cell containing pure water, the beam may be rendered visible before and after its passage through the

water, but no cone of light is seen in the water itself when viewed at right angles to the direction of the light and against a dark background. If now a colloid be dispersed through the water, light will be *diffracted* from the particles in the water and the beam will appear in the solution as a diffuse cone of light. This diffracted light is **plane polarised** (p. 126), and is always produced when light passes through any medium containing particles whose diameter is small in comparison with the wavelength of light.

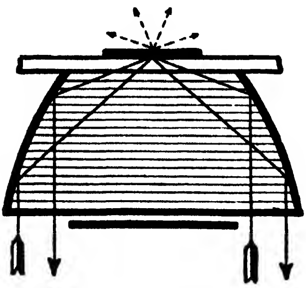


FIG. 15.—Diagrammatic section through a Wenham paraboloid condenser to show the direction taken by the rays of light. (Hatschek.)

(*c*) **The ultra-microscope** is, in principle, just a means of viewing the Tyndall cone through a microscope. A powerful beam of light is thrown horizontally through a small body of fluid placed under a microscope set vertically. The only light entering the objective is that diffracted from the particles present in and optically different from the fluid (Fig. 15). The apparent image bears no relation to the actual size of the particle, but depends on the intensity of the light, and on the indices of refraction of the particle and the dispersant. Nevertheless, by making certain assumptions, the size of the particles may be calculated. The essential feature of the ultra-microscope is not that it is a more powerful kind of microscope, but a new method of illumination, so

TABLE XII

LOWER LIMITS OF DIAMETERS OF SMALL PARTICLES.

Visible under Microscope	Not visible under microscope		
MICRONS $0{\cdot}2\mu$ or $2{\cdot}5 \times 10^{-5}$ cm.	SUB-MICRONS Visible by ultra-microscope		AMICRONS Not visible by U.M. under $1{\cdot}0\mu\mu$
SUB-MICRONS (photographed by U.V. light) $100\mu\mu$ or $1{\cdot}0 \times 10^{-5}$ cm.	Electric arc $15\mu\mu$ or 15×10^{-7} cm.	Strongest Sunlight $1{\cdot}0\mu\mu$ or $1{\cdot}0 \times 10^{-7}$ cm.	

μ equals 10^{-3} mm. $= 10^{-4}$ cm., $\mu\mu = 10^{-7}$ cm.

that ultra-microscopic particles are rendered self-luminous. The conditions under which these small particles can be made apparent by this means are that (1) the light scattered is sufficient in *intensity* and is suitable in *wave-length* to affect the retina ; (2) the particles differ materially in refractive index from their dispersion medium ; and (3) the particles are not so crowded as to overlap.

Particles visible under the ordinary microscope are called microns. Smaller particles are termed sub-microns, if they are rendered apparent by the ultra-microscope ; if not, they are amicrons. The smallest particle of gold observed by Zsigmondy, using bright sunlight illumination, was 1·0 $\mu\mu$ in diameter. Bearing in mind the large difference in index of refraction between gold and water, this may be considered as the smallest particle ever observed. The table on p. 80 (from Zsigmondy) shows the limits of size of the various classes of particles (Table XII.).

Properties of Colloids Depending on the Size of the Dispersed Particles

(ii.) **Kinetic.**

(*d*) **The Brownian Movement.** The little dots of light seen under the ultramicroscope are not at rest. They dart about hither and thither in a seemingly inexplicable way. According to the kinetic theory of matter, a fluid is assumed to be made up of molecules in a state of very rapid motion and having a mean free path intermediate between that of a solid and that of a gas. The colloidal particles in the liquid are hustled into motion by continuous collision with the rapidly moving molecules, of the liquid. If the particles have a natural period of vibration which is a multiple of that of the water molecules, their amplitude of vibration will be increased (*e.g.* by suitably timing blows on a pendulum its excursion can be increased to a considerable extent. Each blow need be very slight).

This motion of the particles, while a very striking feature in the field of vision of the ultramicroscope, is not specifically characteristic of colloidal solutions. Particles sufficiently small to be influenced by the high velocity bombardment of the molecules or ions of the solvent may still be well within the limits of visibility under an ordinary microscope. This movement owes its name to its discoverer Brown, a botanist, who described the peculiar oscillation of pollen grains suspended in water in 1827. This Brownian movement may be seen by means of an ordinary microscope in a suspension of the water-colour gamboge, especially when the diaphragm of the microscope is almost closed. The rate of move-

ment is independent of the chemical nature of the particles, but depends on three factors, viz. (*a*) the size of the particle, (*b*) the temperature, and (*c*) the viscosity of the dispersion medium. The rate is increased by decrease in the mass of the particle, by increase in temperature or by decrease in the viscosity of the medium. The movement persists, never changing, once equilibrium has set in. It has been observed in granite and in other rocks in small pockets of liquid, which they must have occluded for millions of years.

Direct observation of the absolute motion of the particles is very difficult, although differences in motion are easily perceptible. This difficulty has been overcome by the application of the cinematograph to the microscope. A glance at Fig. 16, obtained

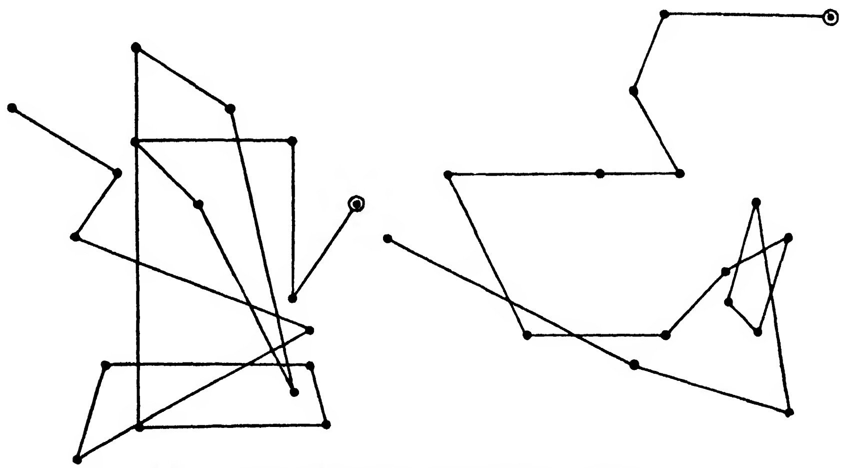


FIG. 16.—Movements of two particles of india-rubber latex in colloidal solution, recorded by cinematograph and ultramicroscope. (Henri.)

in this way, shows that a particle oscillates apparently in a haphazard fashion about a certain mean position during a short interval of time. Any alteration in the kinetic energy of the dispersing medium, of course, produces alterations in the mean velocity of the particles—*e.g.* increase of temperature increases velocity. When the viscosity of the colloidal solution is increased by the formation of a gel, the particles aggregate in one way or another, their mean free path is reduced, and consequently their motion is reduced in amplitude ; the resistance to movement is increased, and so their velocity may become smaller and smaller till they stop altogether. This phenomenon has been studied in order to find out something about the structure of gels and will be referred to later. The velocity may also be modified by alterations in the hydration of the particles. Ramsay considers that the

particles in pure water do not touch one another at any time, each particle being surrounded by a liquid layer. This layer is destroyed by the addition of salts.

To use a somewhat homely illustration, the colloidal particle may be likened to a morsel of bait dropped into the water of a river estuary. The moment that it reaches the water it is pushed to and fro by a multitude of hungry small fish. The velocity and amplitude of the oscillatory movements of the bait depend principally on the size of the bait and on the energy with which it is attacked.

(*e*) **Distribution of Particles.** If a fine suspension of gamboge or mastic be kept undisturbed at constant temperature for some time, *Perrin* found that there was a distribution of the particles under the influence of gravity. At the bottom of the container will be found a denser distribution than at the higher levels. This is exactly similar to the decrease in the density of the atmosphere with height above sea level, and Einstein argued that the distribution of suspended particles with height should follow the law which governs the density of the atmosphere with height. Perrin proved by experiment that this was true. In one experiment with mastic at four different levels 12μ apart he found 116, 146, 170 and 200 particles per unit. For the same levels the following values were calculated: 119, 142, 169, 201. After this adjustment of concentration to level has been reached, no other change seems to take place. While this is true for the comparatively coarse suspensions used by Perrin, and even for finer suspensions *when examined in very thin sheets of liquid*, it has definitely been proved to be untrue for colloids, and even for emulsions when the portions of the liquid within about 100μ of a surface are neglected. In the body of the liquid gravity seems to play no part in the arrangement of the particles, the concentration being uniform throughout the non-surface portion and remaining so for an indefinite length of time. This stability is due to the electrical properties of the dispersed material.

Study of the optical properties of colloidal solutions leads one to the conclusion that the individual dispersed particles, although they are too small to be *seen* by the microscope under any power, *i.e.*, cause no obstruction to the larger light waves, are still able to cause deviation of the ripples of light. One would, therefore, expect that they would show colligative properties indicative of more sluggish particles than those in simple molecular solution. Briefly, their osmotic pressure, activity of diffusion and power of lowering the vapour pressure of water would be low, and their viscosity and their resistance to the passage of salts through them

would be high. However, definite statements like that cannot be made generally about colloids. They are true of physiological colloids, especially of those in gel form, but are not all applicable to the suspensoids.

(*f*) **Ultrafiltration.** Evidence tending to confirm the limits of size found by optical methods of investigation is afforded by experiments initiated by the classical series of ultrafiltrations of Bechold. Membranes of known permeability are prepared, *i.e.* the diameter of the pores is known, and the colloidal solution is filtered through these by pressure. A series of filters is tried till one is obtained which has the smallest pores which will allow the colloid to pass through. Obviously the particles must be smaller than the pores, and also, equally necessarily, they must be larger than the next filter in the series. The sizes of particles obtained in this way are in reasonable agreement with the values obtained from ultramicroscopic calculations.

TABLE XIII

SIZE OF PORES IN FILTER PAPER

(*H. Bechold and R. Lucas.*)

Number and type of paper.	Size of pores in μ approx.
1,450	4·8
598 thick filter paper	3·3
597	2·9
602 (hard)	2·2
(baryta filters)	
566	1·7
602 (extra hard)	1·5
Chamberland—Kerze	0·2–0·4
Reichel—Kerze	0·16–0·18

(*g*) **Osmotic Pressure.** Pure colloids in neutral water have a very low osmotic pressure. This is just what one would expect when one remembers that the osmotic pressure of a solution is related to the number of particles dispersed in unit volume. The value of the osmotic pressure of suspensoids is very small, and seems to vary experimentally with the method of preparation of the colloid. Hydrophilic colloids, both sols and gels, have a measurable osmotic pressure, *e.g.* a 1·25 per cent. solution of pure egg albumin (Lillie) gives a pressure of 20 mm. of mercury at room temperature. It is very difficult to prepare pure hydrophilic dispersoids, because of the way in which they retain crystalloids, and the presence of these salts materially modifies the osmotic pressure of the colloid. This modification is not simply additive as it would be if salts were

added to a molecular dispersion, but varies with the salt used. Some salts cause an increased and some a decreased osmotic pressure to develop. In general, electrolytes belong to the latter class, the extent of their depressing influence depending on the nature of the ions composing the electrolyte. As regards the anions, there is a definite order of increasing depressing power as one passes along the series

$$CNS < I < Br < NO_2 < Cl < SO_4$$

Similarly the cations may be arranged as follows : alkali metals < alkaline earths < heavy metals.

The hydrogen ion concentration of the whole colloidal system has a very great influence on the nature of the osmotic change produced by any added salt.

(*h*) **Diffusion.** The large size of the colloidal particles, especially those of hydrophilic sols, prevents their rapid diffusion through still water. In fact, coloured colloidal substances can readily be arranged in the order of increasing size of the dispersed phase by noting the rate with which the colour passes into water in a test tube.

Diffusion of Salts.—Dissolved substances diffuse easily into or out of gels, the rate of diffusion depending on the concentration of the diffusing substance and on the nature of the colloid. Resistance to the passage of the diffusant varies from gel to gel according to their *structure* and *viscosity*. The obstructive power of a gel may be altered by alterations of temperature, which alters both the kinetic energy of the diffusing salt and the viscosity of the gel. Some colloids, like albumin, develop a definite semi-solid structure when heated, while others of a gelatin nature become more liquid.

The rate of diffusion may be altered by the addition of certain substances to the gel. A gel, after treatment with sodium sulphate, glucose, alcohol, glycerol, etc. (dehydrating agents) offers considerable resistance to the diffusion of electrolytes. Urea, iodides, and chlorides, on the other hand, cause acceleration of the rate of diffusion. These added substances cause alteration in the relative amounts of water held by dispersoid and dispersant and so produce alterations in the more liquid phase. The degree of continuity of liquidity is a causative factor in the velocity of diffusion.

The Cause of Diffusion.—What is the force that drives the solute to all parts of a solution or of a sol or of a gel so that except at interfaces it is equally distributed throughout the mass ? Just that force which causes a gas to diffuse equally through a container

and which causes a solute to exert osmotic pressure, viz., the kinetic energy of the particles (ions, atoms, molecules, or larger aggregates).

ELECTRICAL DIFFUSION.—The rate at which electrolytes diffuse into gels may be increased by the passage of an electric current. This method is sometimes employed in the administration of drugs,—so called ionic medication. "Metal"-ions (cations) are carried into the tissues from the positive electrode of any current-supply device, while "acid"-ions (anions) are driven in from the negative electrode (see Chap. XI. and Part II., p. 529).

FIG. 17.—Adsorptive stratification of silver bichromate in an agar gel. (Bradford, *Biochemical Journal*.)

LIESEGANG PHENOMENON.—If a gel contains a substance in solution and a second substance capable of reacting with the first is allowed to diffuse into the gel, the product of the reaction is deposited in strata separated by clear intervals (Part II.). These banded precipitates were first prepared by* Liesegang in a slightly different form. A quantity of 4 per cent. gelatin sol to which had been added 2 c.c. of a concentrated solution of potassium bichromate was poured on a clean glass plate and allowed to set to a gel in a very thin film. When firm, a large drop of 25 per cent. silver nitrate was placed in the centre of the film, the plate being kept horizontal. After remaining undisturbed (in the dark) for two days or so, concentric rings of silver bichromate were found round the original drop, separated by clear zones free from the precipitate, the distances between the successive rings being greater the further from the centre they are formed. Since the publication of the details of the original experiment many different gels and mutually precipitating salts have been tried. For example, water-glass may be used as medium, or with certain precautions agar-agar, or even a test tube full of a fine powder (flowers of sulphur) or packed with vertically placed capillary tubes. The illustration (Fig. 17) shows beautiful rings of silver-bichromate in an agar gel. A very instructive modification of the

experiment, also due to Liesegang, is to fill a glass tube with a 4–5 per cent. gelatin sol containing about 10 per cent. sodium chloride. When the gel has set, the tube is immersed in a silver-nitrate solution which will diffuse steadily into the gel from both ends, leaving continuous bands of silver chloride. These bands approach one another, but *they do not meet.* A clear space is left in the middle of the tube.

The explanation of the phenomenon seems to be that as the silver ions (of the original experiment) diffuse into the gel they meet the bichromate ions, and some of the silver forms silver bichromate and is precipitated. Now, as the diffusing ions move into the gel in straight lines (Reigel and Widjoff), and as conditions are similar for the whole first line of advancing ions, they will undergo precipitation practically simultaneously and form a layer of silver bichromate in one plane. By the formation of the layer a certain amount of gelatin has lost its bichromate. Diffusion of bichromate ions will, therefore, occur to fill this gap. At the same time, silver ions are advancing outwards and being precipitated. These newly formed precipitates are at first attracted to the first ring and adsorbed, so increasing the thickness of the ring. On account of the greater concentration of the invading ions, they advance more rapidly than the diffused solute and so pass beyond the first ring, and after crossing a space with too low a concentration of opposing ions to cause combination to take place, again form a ring of precipitate, and so on. By the time that the silver has reached the periphery of the plate, its concentration and, therefore, its diffusion rate have become very low. The rings will, therefore, have a longer time, *i.e.* greater opportunity to adsorb the solute, and so they will be heavier and more widely separated.

Dialysis.—If a tube open at both ends and filled with a gel is placed so that one end is immersed in water and the other end in a solution, it will be found after a time that a considerable quantity of the solute will have passed through the intervening colloid and be distributed in the water. If sufficient time were given, the concentration of the solute in the water at both ends of the tube would become equal. On the other hand, a colloid sol placed in the solution would not have diffused appreciably into the gel. This gives us a method of separating colloids from salts. The thickness of the intervening gel is reduced to that of a thick film, and instead of using for this purpose a gel soluble in water like gelatin, a water-holding substance like collodion is employed. Instructions are given in Part II. for the preparation of various types of dialysers, *i.e.* pieces of apparatus, principally consisting of a film of collodion or a piece of parchment which can be used to

separate colloids and crystalloids from a mixture of these two constituents. If instead of keeping one side of the dialysing membrane immersed in water, running water is substituted, or the water is changed often, the colloid in the dialyser can be freed from practically all the crystalloid mixed with it. Fig. 18 is an illustration of Abel's vividiffusion apparatus, by means of which crystalloids—salts, glucose, amino acids, etc., can be removed from the colloids—albumin, globulin, fibrinogen, etc., of the circulating blood. It consists of a number of collodion tubes in parallel, which may be interpolated between the two ends of a cut artery in an anæsthetised animal so that they are functionally part of an intact circulation. Now, as have we seen, a diffusible substance will pass out into the surrounding water in the glass container, at a rate depending on the difference in the concentration of that solute on both sides of the membrane (Fick's Law). If we want, say, to study the amino acid content of the circulating blood, all we have to do is to arrange matters so that our outer liquid starts with a concentration of no amino acids and a concentration of all the other diffusible substances of blood equal at least to their concentration in the blood.

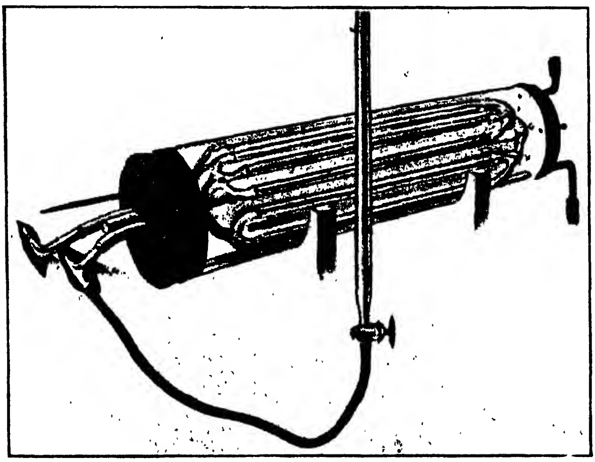


FIG. 18.--Abel's vividiffusion apparatus.

(*i*) Viscosity. It is obvious that some liquids offer a greater resistance to stirring than others. Water and all true solutions in water, even fairly concentrated ones, differ little from one another in this respect. Even if small particles are suspended in the water to form a suspensoid, or larger particles such as a precipitate of barium sulphate in suspension, the additional resistance to shearing is not very great. But when one comes to deal with hydrophilic sols, and more so with gels, considerable force is required to push a stirring rod through the liquid, *i.e.* the viscosity of hydrophilic colloids is much greater than that of water, *e.g.* at 38° C. water has a viscosity of $6{\cdot}6 \times 10^{-3}$ dynes per sq. cm., and blood serum about twice that amount, viz. from $9–12 \times 10^{-3}$ dynes per cm^2.

The value of viscosity is of some importance in the study of the circulation of the blood, because, if the resistance to the movement of the blood in the capillary vessels is increased, the heart will have to expel the blood at a greater pressure to force the fluid round the circuit. Several methods have been employed to measure this value. The one most commonly used is to measure the rate of flow of a measured quantity of the fluid under test down a vertically held capillary tube under standard conditions. This rate, after correcting for density, is usually compared with the rate obtained under the same conditions for an equal quantity of water (Part II., p. 530). Sometimes it is desirable to determine the **coefficient of viscosity** in C.G.S. units. The coefficient is defined as the tangential force per cm.^{2} on either of two horizontal planes 1 cm. apart, one of which is fixed while the other moves at 1 cm. per second, the space between being filled with the liquid under test. Hatschek used for this determination a piece of apparatus consisting essentially of two concentric cylinders, the outer one of which can be rotated at any desired rate while the inner one is suspended from a wire. The liquid fills the space between the cylinders. When the outer cylinder is rotated it carries with it the thin layer of liquid in contact with it. This liquid layer in turn pulls at the layer next to it, and so on till we come to the almost stationary layer in contact with the inner cylinder. That is, we may consider the fluid between the cylinders to be made up of a number of concentric liquid cylinders, each exerting a certain fractional force on the adjacent cylinders. The degree of torsion of the suspending wire gives a measure of the viscosity of the liquid.

The main factor on which the large value of the viscosity of hydrophilic colloids depends is that the shearing force has to overcome not only the internal resistance of the liquid continuous phase, but the resistance to distortion of the elastic colloid dispersed phase.

CONCENTRATION.—The latter resistance, of course, increases with the number of colloid particles encountered by the distorting force *i.e.*, on concentration. Up to a certain concentration limit, which varies with different colloids, increase of concentration makes very little difference in the value of the viscosity. Above this limit, a very sharp increase of viscosity occurs. One may take this limiting value as a measure of the hydrophilic properties of the colloid, *e.g.* *caseinogen* starts to increase its viscosity markedly at about 5 per cent., while *glycogen* goes up to 25 per cent. before being effectively viscous.

TEMPERATURE.—Alteration of temperature produces marked and regular alterations in the value of the viscosity of pure water, *e.g.*,

about 2 per cent. per degree. With hydrophilic colloids it varies with the colloid in both degree and sign, *e.g.* gelatin is less viscous while albumin is more viscous at 60° than at 10° C.

Hydrogen ion Concentration.—At the isoelectric point (see p. 92) physiological colloids have their lowest viscosity. Any increase of H^+ above this point leads to increased viscosity. Decrease of H^+ below the isoelectric point also increases viscosity, but not so markedly. This increase of viscosity with altered pH soon reaches a sharp upper limit. Any further alteration of pH produces a decrease in viscosity due to a dehydration of the colloidal micelle.

Salts.—The effect of salts on the viscosity of a colloid at its isoelectric point is very slight. It is found that the addition of salts to an acid or to an alkaline protein tends to reduce the viscosity to that found at the isoelectric point. The various neutral salts differ in the intensity of their power to antagonise acid or alkali in colloids, depending principally on their valency (see Part II.).

2. PROPERTIES OF COLLOIDS DEPENDING ON THEIR ELECTRIC CHARGE

We have used terms in the discussion on viscosity above which indicate that colloidal particles carry an electric charge. By virtue of this charge the particles of the disperse phase will act like ions and will migrate through the solution to any point of opposite charge. This electrical migration is called **cataphoresis** (Figs. 19 and 20). If, on the other hand, the colloid cannot move, say it is in gel form or associated with a membrane impermeable to it, then the molecules of the water in which it is immersed will move relative to it. (See Electrical Endosmose, p. 142).

The charge on colloidal particles may be developed (*a*) electrostatically, (*b*) by orientation of the molecules on the surface of the colloid, or (*c*) by some adsorption effect. Most inert substances when immersed in water collect a negative charge, while a very few become positive. This is true whether we are dealing with particles of colloidal size or with large masses, *e.g.* basins, beakers, etc., and is generally considered as due to *electrostatic* causes, *i.e.* the surface picks up electrons liberated by the energy of agitation of the water molecules (ionisation, *q.v.*). Many colloids are amphoteric, *i.e.* can give rise as occasion offers to either + or − " ions." That is, they will have two ionisation constants—an acid one and a basic one. Now if an ampholyte is immersed in water and a *strong* acid added, the ionisation of the weak acid of

the colloid will be repressed, and so the colloid will appear basic, *i.e.*, will act as if composed of cations. On the other hand, treat-

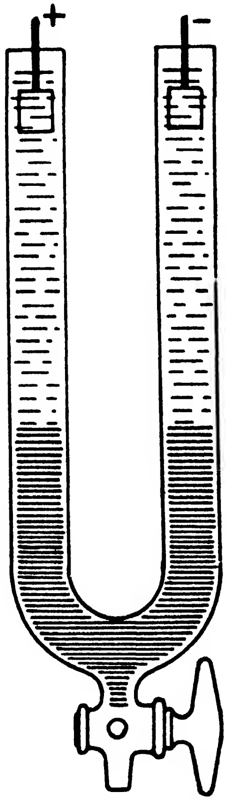


FIG. 19.—Apparatus for demonstrating cataphoresis. The deeply shaded lower portion of the U-tube is filled with a colloidal sol, the upper part with ordinary distilled water. On the passage of an electric current the colloid rises towards the electrode of opposite sign to the sol. (Hatschek.)

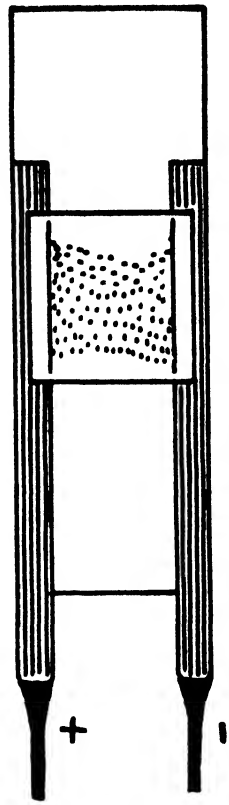


FIG. 20.—Apparatus for ultramicroscopic observation of the movements of colloids in an electric field (see Part II.). (Hatschek.)

ment with a *strong* alkali will give the colloid acidic or anionic properties. Hardy (1899) noticed that the particles of egg albumin

TABLE XIV

ISOELECTRIC POINTS OF COMMON PROTEINS

Animal.		Vegetable.	
Nucleoprotein (Pancreas)	3·52	Glutenin	4·45
Serum Albumin		Edestin	4·5–8
Casein	4·7	Gliadin	9·0
Gelatin			
Egg Albumin	4·8		
Fibrinogen	5·0		
Serum Globulin	5·4		
Oxyhæmoglobin	6·74		

sol migrated to the cathode in acid solution or to the anode in alkaline solution.

Isoelectric Point.

At a pH of 4·8 in Hardy's cataphoresis experiment the albumin particles did not migrate to one pole or the other. This indicates that the protein is equally ionised as an acid and as a base, and is electrically neutral. The isolectric point of an amphoteric colloid is that pH at which certain of the characteristic properties of the colloid are minimal, *viz.*, osmotic pressure, viscosity, imbibition and stability, as is shown for gelatin in Fig. 21 from Loeb's results.

The actual concentration of H ions at which these minimal values are reached is specific for each colloid. Most animal proteins are isoelectric on the acid side of neutrality, while vegetable proteins reach this point on either side of pH 7.

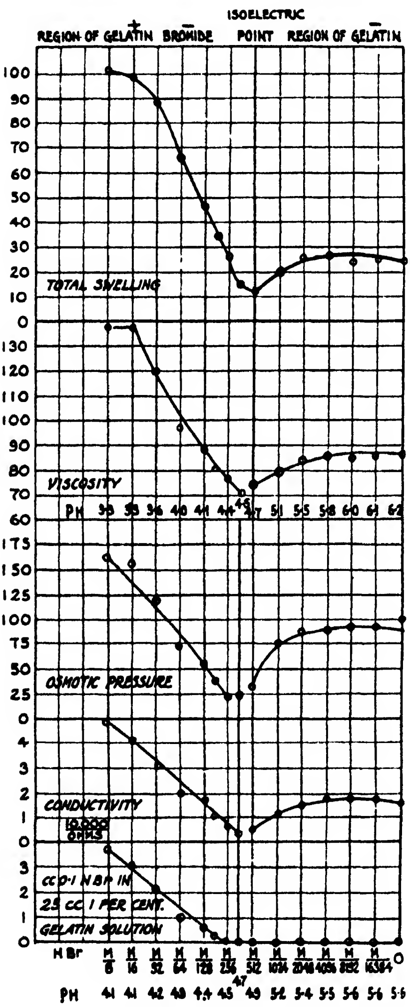


FIG. 21.—Curves showing that the total swelling, viscosity, osmotic pressure, and conductivity of gelatin are minimal at the isoelectric point, p_H 4·7. (After Loeb.)

Coagulation of Gels and Precipitation of Sols.

As we have seen, at the isoelectric point, the colloidal particles become electrically neutral, and, therefore, one of the factors tending to keep them apart has been removed, *viz.*, the repulsion of similarly charged bodies. When, by simple molecular agitation, some of the particles pick up an electron, as they are bound to do, and so become differently charged from their neighbours, mutual attraction takes place; these particles coming together form larger aggregates, thus accounting for the lowering of osmotic pressure and of the stability of the dispersion at this point.

This coagulation may be brought about by adding (*a*) acids or alkalies, (*b*) suitable electrolytes, or (*c*) colloids of opposite sign. The coagulation of suspensoids (Class IV. colloids) by the above means is easily carried out and is a reversible process. On washing out the adsorbed precipitant the dispersoid is re-established. Hydrophilic colloids, on the other hand, are more stable than hydrophobic colloids. They usually need the addition of a large quantity of the coagulating substance and the resulting coagulum is frequently irreversible. We have seen that they are diphasic systems where the continuous phase is more or less a continuation of the disperse phase. If a substance A is dispersed in water to form an emulsoid, what really results is a dispersion of a solution of water in A, throughout a solution of A in water. The stability of such a system will depend in great measure on the viscosity of the intermicellar liquid. The viscosity depends on the concentration of the more viscous A in the less viscous water. The range of viscosity making for stability will be bounded on the one hand by a certain minimum and on the other hand by a certain maximum concentration of water in the continuous phase.

Salting Out.

There are two factors implicated in the precipitation of hydrophilic colloids by salts, one of these (α) is determined by the degree of hydration of the colloid (see p. 98), while the other (β) is related to the solubility of the colloid-hydrate at the isoelectric point. At this pH, the two factors may be included in the equation

$$\log s = am + \beta_0$$

where s = solubility of the colloid and m the molecular concentration of the salt. If precipitation is not carried out at the isoelectric point, but at some other hydrogen ion concentration, then instead of β_0 another constant β_x has to be used, depending on the pH. If the precipitations are not induced at the isoelectric point of the colloid, the powers of various salts to flocculate any colloid depends on the valency of the *cation* if the pH of the fluid is greater than the isoelectric pH, and on the valency of the *anion* if the solution is on the acid side. That is, under these conditions only those ions are effective coagulants whose electric charge is opposite to that of the colloid (Hardy's rule).

Protective action of Hydrophilic Colloids.

Many emulsoids when added in comparatively minute quantities to suspensoids prevent the coagulation of the suspensoids by electrolytes. As a matter of fact, each emulsoid which exhibits this property has a characteristic protective power which may

be used as a definite factor for the identification of the colloid. The suspensoid generally used in the test is colloidal gold. Zsigmondy, who devised the method, defines the "gold number" as the number of milligrams of an emulsoid which are just sufficient to prevent 10 c.c. of a bright red gold sol (prepared under certain specified conditions) from changing into violet or shades of violet after the addition of 1 c.c. of 10 per cent. sodium chloride solution (Part II.).

He divides colloids into four classes according to their "gold number," viz.:

TABLE XV

Class.	Gold Number.	Examples.
I.	0·005–0·1 mg.	Gelatin, caseinogen, animal glue.
II.	0·1 –10 mg.	Crystalline egg albumin, gum acacia.
III.	10 –500 mg.	Dextrin, starch.
IV.	Inactive–	Mucin, silicic acid.

The general opinion seems to be that the emulsoid forms a pellicle round each suspensoid particle and prevents coagulation, either (1) because, as we have seen, emulsoids are less sensitive to the precipitating action of electrolytes and the compound particle is endowed with an emulsoid coat; (2) because the electrolyte does not come in contact with the suspensoid particle and does not neutralise its electric charge, or (3) merely by offering a material obstacle to the coalescence of the particles.

The fluids of the body contain two colloidal substances of peculiar interest. Albumin and the globulins are emulsoids, but they differ physically in at least three respects summarised below.

TABLE XVI

Albumin.	Globulin.
Sol in water.	Insol in water.
(Sol in 5 per cent. NaCl.)	(Sol in 5 per cent. NaCl.)
Not ppt. by ½ sat. $(NH_4)_2SO_4$.	Ppt. by ½ sat. $(NH_4)_2SO_4$.
Protects suspensoids.	Ppts. suspensoids.

Albumin has a protective action on gold sol, while globulin acts almost as if it were a suspensoid. The proportions of albumin and globulin in the various body fluids are practically invariable in health, but during the course of various diseases the balance is upset. If the globulin content is increased relatively to the albumin, then the body fluid will lose a portion of its protective

power. In some cases the globulin is not increased, but carries an increased positive electric charge. This increases its precipitating action. (Method given in Part II.)

Precipitation by Colloids of Opposite Charge.

Colloids that are present together in the same medium may mutually precipitate one another, either because being of opposite sign they neutralise their charges, or because they sensitise each other to electrolytes. As an example of the former action we may consider the usual method of getting a protein-free filtrate from serum by the addition of a calculated quantity of colloidal iron or of tannic acid. The serum proteins are, as found, slightly alkaline, and carry a small negative charge. This is neutralised by the positive charge on the iron or on the tannin (Part II.).

A similar method could not be used for whole blood because the hæmoglobin at pH 7·4 carries only a feeble negative charge and would very readily take on a + charge. A small quantity of colloidal iron is added which distributes itself over the blood colloids. If one now precipitates the *liq. ferri oxidati dialysati* by an electrolyte to which it is sensitive, *e.g.* K_2SO_4, it will rapidly separate out, carrying with it the blood proteins. This is an example of the second type of mutual precipitation.

A special instance of this kind is found in the precipitation of electro-positive dyes on filter paper,—colloidal cellulose with a negative charge in water, intensified by the presence in it of electrolytes, especially calcium silicate (Part II.).

Of great interest in this connection is the reaction of proteins to dyes. In histological technique, various " basic " and " acidic " dyes (see p. 53) are used to obtain a differential staining of various tissues or to indicate cell structure. Gortner has found that at physiological concentrations of hydrogen ions the dye combines *chemically* with the protein, the dye anions forming a salt with the protein cations, and *vice versâ*. For example, the nuclear material contains a predominant amount of acidic protein and so attracts dye cations. If the cation of the dye is coloured, *i.e.* if the dye is *basic*, it will stain the nucleus. On the other hand, at greater acidities (pH 2·5–1) true adsorption takes place with a neutralisation of the electro-kinetic potential on the surfaces of the colloids. That is, at hydrogen ion concentrations not far removed from the isoelectric point of the proteins the amount of dye fixed is determined by the chemical composition of the proteins, and differs, of course, for the various proteins concerned. When the deviation from the neutral point is greater, more dye is taken up by adsorption. This extra amount is independent of the chemical composi-

tion of the proteins, and is determined only by their concentration and charge. Such stains as Van Gieson's depend on this reaction.

Action of Radiant Energy. (See also Chap. XIII.)

The intimate connection between coagulation and the charge carried by the particles is shown by the action of the β rays of radium. As these rays are negative charges of electricity, they should stabilise negative colloids by increasing their charge, and precipitate positive colloids by neutralising their charge. Hardy found that positively charged acid-globulin was reduced to a state of jelly in three minutes, while the particles of negatively charged alkaline-globulin were rendered more mobile by exposure to β radiation. Schanz found that ultra-violet light by its power of ionising water could decrease the solubility of albumin so that it was precipitated along with the globulins. He attributed the production of sclerosis and lack of elasticity of the lens of the eye to light of short wave-length acting in this way on the mixture of albumin and globulin composing it.

Heating and Cooling, which alter viscosity directly and also indirectly by altering the amount of water distributed between the two phases, also cause coagulation. Heating certain *sols* changes them into the more rigid *gels*. Various native proteins, for instance, those of egg white, serum, muscle, coagulate to a gel on heating to a temperature specific for each protein. This process is irreversible and takes place in the presence of electrolytes. On the other hand, gelatin forms a sol on heating and a gel on cooling—a reversible reaction which is profoundly modified by the presence of electrolytes.

3. PROPERTIES DEPENDING ON SIZE, CHARGE AND STRUCTURE

Adsorption. Adsorption to a surface was considered in Chap. VI. Colloids are characterised by their large *specific* surface, by the development of surface charges due to this surface, or, in some, to their amphoteric nature, and in the case of gels, by an internal structure offering a surface to the dispersion fluid. If the colloidal particles are free to move, *i.e.*, if the colloid is in sol form, adsorption may take place in either of two ways. The colloid may be adsorbed to a surface or matter may be adsorbed to the colloid. Dyeing with colloidal dyes offers an example of the first kind, while the fact that natural colloids are always impure on account of adsorbed mineral matter indicates the truth of the latter statement. The gels with their internal structure present peculiar surface properties. They have a special propensity for adsorbing their dispersion

medium (see below, Imbibition). In all cases of adsorption the chemical and physical properties of the adsorbed material are altered by the process. Not only are the adsorbed molecules oriented, but they are held under compression. It is in these condensed layers that many typically physiological reactions take place—reactions which could only occur with great difficulty in dilute solutions (Chap. X.). Further, the adsorbed salts are rendered for the time being osmotically inactive. They are removed from any active part in the solution.

The process of liberating the adsorbed material has been dealt with in a previous chapter (VI.). The result of the process of de-adsorption is the restoration of the physical properties of the adsorbed material. We shall see later (Blood) when, how and with what effect adsorption and de-adsorption take place.

Imbibition. The adsorption of water is of such biological importance that it requires special consideration. All the physiological colloids have the property of taking in relatively large quantities of water even against enormous pressures, and of holding this water against even strenuous methods of removal. This "bound" water stored in the micropores (p. 55) is under considerable compression, so much so that its density and all its physical properties are altered. A very instructive demonstration of this, due to Du Bois Reymond, is given in a modified form on p. 537. A piece of the seaweed *laminaria digitata*, which can be bought dried and ready for use under the name of *tangle tents*, is attached by a thin copper wire to a piece of cork of such a size that it just floats in water (*i.e.* the system cork-wire-laminaria has a density of 1 approx.). The following table gives the results of its immersion in water.

TABLE XVII

COMPRESSION OF WATER IMBIBED BY *LAMINARIA*

Days.	w_1.	w_2.	$v = w_1 - w_2$.	$d = \frac{w_1}{v}$.	$\frac{\text{Increase in } w_1}{\text{Increase in } v}$.
0	2·64	0·83	1·81	1·46 ± 2	0
1	12·02	0·75	11·27	1·09 ± 2	0·992 ± 6
3	18·95	0·84	18·11	1·047 ± 2	1·001 ± 3
4	19·67	0·88	18·79	1·047 ± 2	1·003 ± 3
5	19·93	0·92	19·01	1·049 ± 1	1·005 ± 4
6	20·12	0·92	19·20	1·048 ± 2	1·005 ± 4
8	20·27	0·91	19·36	1·047 ± 2	1·005 ± 3
10	20·31	0·91	19·40	1·047 ± 2	1·005 ± 3

W_1 = Weight of Laminaria in Air.
W_2 = Weight of Laminaria in Water.

Laminaria balanced with cork and wire on zero day. On the first day it floats, on the second it is as on zero day, and on the third day it sinks. If more cork is now added so that the system just floats again, the laminaria will sink again on the fifth day.

If w = wt. of cork and wire, v = vol. of cork and wire.

Then on zero day, $w + 2{\cdot}64 = v + 1{\cdot}81$ (*i.e.*, density = 1)

$$\therefore v = w + 0{\cdot}83.$$

Then on any day (say 10th)

Density of whole system

$$\text{is } \frac{\text{wt.}}{\text{vol.}} = \frac{w + 20{\cdot}31}{v + 19{\cdot}40}$$

$$= \frac{w + 20{\cdot}31}{w + 20{\cdot}23} \text{ which is} > 1.$$

Hence on the 10th day the laminaria will have sunk if just balanced on the zero day.

It will be seen that at first the density of the system decreases

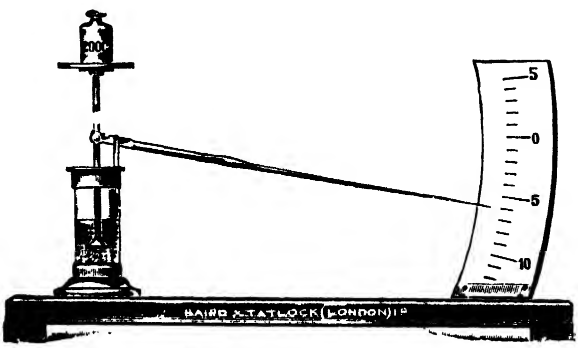


FIG. 22.—Œdometer for determining the swelling pressure of colloids.

slightly, and then rapidly increases—the system sinking in water. The primary decrease, occurring when the macropores are filling and the seaweed swells, is difficult to explain and is not relevant to this discussion. During the period of increasing density, water is being *packed* into the micropores in the interior of the gel. This water is " bound " and can only be driven off by the application of suction and heat. Many similar experiments have been devised to show the same phenomenon. In Fig. 22 is an apparatus designed to measure the swelling pressure of seeds or of powdered gelatin. (See also Part II., p. 537.)

The " bound " portion of the imbibed water which is held so fiercely, and which may be so increased in density that it occupies about 75 per cent. of its bulk at atmospheric pressure, has no appreciable vapour pressure at ordinary temperatures (Gortner). It will, therefore, freeze with great difficulty, and if it

does, it forms such small crystals of ice that the colloid structure is not destroyed. Although it is so difficult to drive off, that a silica gel, for instance, can only partially be dried in a vacuum at 300° C., about 5 per cent. of the water remaining after 6 hours' heating (Neuhausen and Patrick), and an alumina gel cannot be dried by heating for 2 or 3 days at 500° C., yet some relationship does exist between the "free" and the "bound" water. Under certain conditions, as yet undefined, bound water may become free again, and the reverse. Many physiological processes may depend on an equilibrium between free and bound water. For example, certain enzymes proceed towards synthesis under one set of conditions and towards hydrolytic splitting under the opposite set. The former conditions are generally admitted to be when the reacting substances are concentrated, *i.e.*, when by imbibition colloids have removed water from the sphere of activity, and the latter when dilution takes place.

Heat of Imbibition (p. 55). During the process of compression a considerable amount of heat is set free. If the swelling takes place relatively slowly, as with *laminaria*, it is not easy to demonstrate the development of heat, but, in the case of colloids which rapidly imbibe water, even such a value obtained by merely stirring the colloid with a thermometer during the process of imbibition is appreciable (Part II.). The amount of heat developed depends on various factors. At the isoelectric point the main factor is the amount of compression of water produced.

TABLE XVIII

PRESSURE AND HEAT OF IMBIBITION OF HYDROGELS

Gel.	Compression (atmos.).	Gram calories per gram of gel.
Dry Gelatin . .	over 300	5·7
Dry soluble Starch .	over 2,500 (Rodewald)	6·6
Dry Gum Tragacanth .	over 400	10·3

It is obvious from Table XVIII. that other factors besides compression play a part. Starch always exerts a large osmotic pull because it is never free from ions. This may, in part, explain the large compression without a correspondingly large evolution of heat.

The amount of water imbibed and the rate of imbibition are lowest at the isoelectric point (Fig. 21). The addition of either acid or alkali greatly increases both rate and quantity. This effect is due to the formation of salts of the colloid, and the colloidal ions so produced exert a pure osmotic pull on the water—the gel itself acting as a semipermeable membrane to its own ions. The

effect of a very *slight* increase in pH on swelling is much more pronounced than a larger increase—*e.g.* 1 gram of gelatin at pH 4·7 imbibes 7 c.c. of water, while at pH 4·4, 21 c.c. water will be taken up. Increasing the hydrogen ion concentration still further produces a fairly steady increase in swelling power till at pH of 3·4 about 35 c.c. of water have been absorbed. The extra amount absorbed by increasing the hydrogen ion concentration still further is inappreciable. When acetic acid or similar weak acid is used to acidify the gel atypically large results are produced, due, according to Loeb, to a diminution in the cohesion of the gel brought about by the high concentration of those acids necessary to give a pH of 3·2.

It is obvious from our discussion of the isoelectric point that the addition of salts to a gel will tend to depress its power of imbibition to a value approximating that found at the isoelectric point due to their inhibitory action on the *ionisation* of the gel. Salts cannot exert an osmotic effect of their own if the gel is freely permeable to them (see Diffusion).

Explanations may now be offered as to why a limiting value is placed on the amount of water imbibed by a gel. The force causing swelling, whether due, as at the isoelectric point to pure capillarity, or to the ionised gel exerting an osmotic effect, is opposed by the elastic forces of the gel. The gel molecules or gel ions exert a cohesive force which has to be overcome. In the case of substances like *laminaria*, the cohesion is very great and does not permit the molecules to be forced very far apart. On the other hand, gelatinous substances have their particles separated sufficiently to make the gel soft, and, finally, if water were freely admitted, the gel would become more and more like a sol.

Syneresis. Graham found that if gels were left undisturbed for some time they underwent contraction and expressed a quantity of their dispersion medium containing some of all the colloidal and crystalloidal matter present. This process he called syneresis (coalescence), and it is common to all gels, but in different degree. Typical examples are separation of *serum* from *blood-clot*, *whey* from *curded milk*, and weeping of *agar* slants.

The behaviour of most organic gels is complicated not only by the presence of electrolytes, and by the fact that the content of the intermicellar fluid in electrolytes may be rapidly altered, but also by the fact that the dispersed substance is a mixture of closely related substances. Thus agar-agar, a carbohydrate superficially similar to the protein-hydrate gelatin, consists of at least two substances α and β agar-agar which are mutually convertible under certain conditions. Purified, α agar-agar is prac-

tically insoluble in water. The β form is very soluble in water. On warming some of the former with water it gradually passes into the soluble form and thus goes into solution. Insoluble α particles may be dispersed in larger particles of β + water. They in turn form a true sol with water. Alteration of physical or chemical conditions will therefore alter the relative concentration of α and β. The β colloid protects its α relative from coagulation by thus forming a pellicle round it. Starch—a pseudo-colloid—is a mixture of several carbohydrates of high molecular weight, each of which is capable of taking up a different quantity of water. (See Emulsions, Chap. IX.)

A similar difficulty occurs in attempting to explain the colloidal behaviour of some of the proteins. The globulins offer an interesting and somewhat bewildering field of study. They are insoluble in water, but soluble in neutral salt solutions *in the neighbourhood of their isoelectric points*. In the list of isoelectric points given on p. 91 you will find that *edestin*, a vegetable globulin, is given no definite figure, but a *range* from pH 4·5–8·0. That is, between those wide limits of hydrogen ion concentration, the reactivity of edestin is at its lowest. Somewhat similar figures could be adduced for the blood globulins. They have large molecules, but very few polar groups capable of combining readily with acid or base—too few indeed to carry them into solution under physiological limits of pH. They, therefore, carry the burden of retaining salts, especially sodium chloride, within the body if they are to remain in solution (see Blood).

It has been shown by Starling that the colloids of the blood were the factors determining the volume of the blood, and that their osmotic pull acting against the filtering force of the blood pressure controlled the output of urine, the formation of lymph, etc. Bayliss clearly demonstrated the function of these colloids, especially in the neat balance between albumin and the globulins in maintaining the viscosity of blood. Swelling of colloidal matter in the erythrocyte under the influence of an acid (CO_2) plays a large part in securing efficient oxidation in the body, and adsorption is necessary for the life of the cell (p. 134). Further, colloids may be regarded as *great reservoirs of energy* in the body—

(1) *As colloids have extremely low osmotic pressures they are a suitable medium for the storage of potential energy.* Carbohydrates may be stored as starch or glycogen, both colloids, and changed readily into maltose or glucose, which are crystalloids.

(2) *The salts adsorbed* by a colloid are thus rendered *osmotically inactive*, but may be set free again by alteration of the colloidal electric charge.

(3) *Some colloids imbibe water and compress it.* A hydrated gel (jelly) has therefore a store of hydraulic pressure within it.

Further Reading

E. Hatschek. "An Introduction to the Physics and Chemistry of Colloids." J. and A. Churchill.

CHAPTER IX

DISPERSE SYSTEMS

II. SOAPS AND EMULSIONS

"When we have familiarised ourselves with the physico-chemic and colloid-chemic behaviour of systems of the type water-dissolved-in-x, we shall find ourselves possessed also of the laws which govern the behaviour of protoplasm under physiologic and pathologic circumstances. MARTIN H. FISCHER.

EMULSIONS are systems consisting of two mutually insoluble liquids, one of which is very finely dispersed within the other.

They may be regarded as emulsoids with somewhat larger dispersed particles (microns). The term, as usually employed, has, however, a narrower connotation, the disperse phase being considered as a fat or fat-like substance distributed throughout water in such a way as to remain stable for an indefinite period. Oil and water are two immiscible liquids, and no amount of mechanical mixing will induce them to form a permanent emulsion. It is true that after a prolonged beating of the two together a maximum of 2 per cent. of the oil may be taken up by the water, forming a stable dispersoid. Measurement of the particles, however, demonstrates that they are of the order of sub-microns, and thus a true colloidal system has been formed. An example of this is the condenser water of steam engines, which contains lubricating oil in suspension.

Analyses of natural and artificial emulsions, like milk, bile, rubber, cod-liver-oil emulsion, etc., demonstrate the presence of more than merely oil and water. A colloid or semi-colloid must be present.

If the generic term oil is used to denote any liquid that is not miscible with water, we may note that there are two entirely different types of emulsions, the one being drops of oil suspended in water and the other being drops of water suspended in oil (cf. sol and gel). For example, milk belongs to the former and butter to the latter class. It is important to know under what conditions each of these types is formed. One might at first imagine that the governing factor would be the relative amounts of oil in water, much water and little oil producing the

oil-in-water type and excess of oil over water producing the water-in-oil emulsion. This is not so. The relative amounts of oil and water have nothing to do with it. To understand

TABLE XIX

Emulsion.	Disperse Phase.	Continuous Phase.	Colloids.	Crystalloids.
Milk	Oil 3·8 per cent.	Water 87 per cent.	Caseinogen Albumin Globulin 3·2 per cent.	Lactose and Salts Na_2CO_3, etc.
Bile .	Fats and Lipoids 5·9	Water 77·5	Soap 3·2 Mucin 0·45	
Egg yolk	Fat 35·3	Water 47·2	Protein 15·6	
Ear-wax	Fat 26	Water 10	Potas. Soap 52	Mostly organic, little ash.
Butternut	Water 4·4	Fat 55·1	Protein 23·7	
Human fatty tissue.	Water 15	Fat 82·5	Protein 2·5	
Rubber latex	Rubber 20 and Resin 2	Water 75·2	Protein 2·8	Sugars, K and Ca, etc.
Pharmaceutical Emulsions	Oil 50	Water 50	Egg white Gum arabic Saponin	Sugar Phosphates Carbonates
Lubricating Emulsion	Water 1	Oil 98	Soap 1	

the significance of this, one must examine the function of the colloid.

Some means must be adopted, once the oil has been dispersed, to (*a*) decrease the interfacial tension between the droplets and the dispersion medium so that the dispersed particles will not coalesce, (*b*) confer on the droplets an electrical charge so as to

cause mutual repulsion, and (*c*) mechanically keep the droplets separate. The presence of an emulsoid seems to confer stability on an oil-water emulsion.

Various theories have been put forward to explain why the presence of a hydrophilic colloid permits of the formation of a permanent emulsion and why some stabilising colloids produce a dispersion of oil-in-water while others favour the water-in-oil type.

(1) Quincke, Hillyer, Donnan and Potts are of opinion that the stability is due mainly to a lowering of interfacial tension by a thin layer of the colloid or semi-colloid deposited on the surface of the droplets of the disperse phase. This interphase reduces the surface tension on the film-water interface, confers a charge on the droplets by adsorption, and, by having the remainder of the colloid as an outer phase, provides a medium sufficiently viscous to keep the droplets in suspension. That is, an emulsion is triphasic.

(2) According to Bancroft, a deposition of the stabiliser in accordance with the Gibbs-Thomson principle (*q.v.*) provides the necessary electric charge and confers protection. He adduces as proof the fact that if another surface is brought into competition with the oil-water surface for the stabilising agent, some proportion of the stabiliser will be adsorbed to this new surface. For example, if the emulsion is allowed to stand the air-fluid interface will capture some of the stabiliser, *i.e.* the cream will carry off some of the colloid, and this, we know, it does.

(3) Fischer considers that an emulsion *may* be triphasic but need not necessarily be so. His idea is that a diphasic system is all that is required for stability, *e.g.* oil and a lyophilic colloid in water. A film of the *solvated* colloid is adsorbed to the surface of the disperse phase.

(4) R. E. Wilson is of opinion that the film on the dispersed fluid is really a plastic solid, *i.e.* butter-like.

All these theories have one fact in common, *viz. the nature of the emulsoid determines the type of emulsion produced.* If the colloid is one which is " wetted " by water (hydrosol or hydrogel), and is adsorbed by oil, it (or its solution) will form a film round the oil droplets and give an emulsion of oil in water. On the other hand, if the colloid is dispersed through oil and is adsorbed by water, it will emulsify water in oil.

The oil cannot be dispersed throughout a hydrated colloid until a certain lower limit of water content has been exceeded, nor can it be divided permanently into a hydrated colloid after an upper limit has been passed.

Emulsions are broken through the institution of conditions that are the reverse of those that make for their stabilisation. In other words, a colloid is a suitable emulsifying agent only when it holds a certain amount of water. That amount may vary between an upper and a lower limit. If at any time the water in the system oversteps either of the limits the emulsion will lose its stability and will separate out. The emulsions hardest to break are those where the emulsifying agent is a carbohydrate like gum acacia, starch or dextrin. They hold their water of hydration with avidity. Salts, acids, or alkalies in moderate concentrations, alcohol, chloroform and ether have very little action on them. Milk, an oil in protein emulsion, is very difficult to break. Dilution has little effect and fat solvents do not readily extract the fat. This is probably due to an adsorption effect in which the carbohydrate plays a part as yet unknown. The colloidal material comes to be concentrated on the surface between the oil and the aqueous phase. These protecting films drawn over the oil globules keep them from coalescing even when brought close together and also form a membrane impermeable to fat solvents.

Similarly the colloid in a water-in-oil emulsion must be hydrated. Using soap as his stabiliser, Pickering emulsified 99 per cent. oil by volume in one volume of water. The resulting emulsion was a stiff jelly which could be cut with a knife and the cube so prepared would stand alone. These solid cubes when left standing in dry air seem to liquefy. The reason for this is that the soap film loses moisture by evaporation, cracks, and sets free the oil. The mass does not become liquid because of the adsorption of water but because of the loss of water. Several of the heavy lubricating oils contain a considerable quantity of calcium soap. Now, calcium soaps are very insoluble in water but form colloidal solutions in oil, therefore, in these lubricants the water is emulsified into the oil and a thick grease is formed. Rosin acts similarly to calcium soap and is used in the preparation of cheap brands of ready-to-use paints as an instrument for the emulsification of water in the linseed oil. As much as 80 per cent. water may be absorbed in this way.

Soaps. Of great physiological interest are soaps, the alkali salts of the fatty acids. These soaps are found in the body wherever fats are found—in bile, blood, fæces, ear wax, sebum, etc., as well as in some pathological fatty secretions. The soaps furnish a series in which the molecular weight regularly increases. Step by step with this increase in molecular weight there is a regular gradation of the properties of the dispersoid from the true solution of the

soaps of the lower fatty acids to the colloidal gels of the higher homologues. This is largely due to the steady increase in water-holding capacity with increase in the length of the carbon chain. The sodium soaps of the *acetic series* show this gradation in imbibition very well. For instance, a gram-molecule of the sodium soap of *caprylic acid* can hold 200 c.c. of water while that of arachidic acid is capable of imbibing 37,000 c.c.

Still more important physiologically is the effect of altering the cation. Sodium, potassium, ammonium, calcium and magnesium soaps are found in physiological analyses and these differ from one another, especially in their power to *hold water*. Ammonium and potassium soaps are so hydrophilic that they do not solidify but form jellies (soft soap). Sodium soaps also hold a considerable amount of water, but only about $\frac{2}{5}$ of that held by "soft" soaps. So little water is held by the soaps of calcium and magnesium that they do not form a sol to any appreciable extent. The addition of alkali to a sodium soap greatly increases its hydrophilic properties.

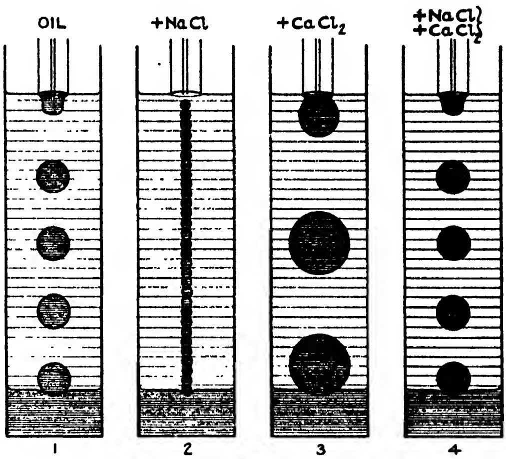


FIG. 23.—To demonstrate the effect on the number and size of drops formed from a given volume of olive oil dropping from a standard tip through solutions of 0·001 N, NaOH alone (tube 1), and plus 0·15 N, NaCl (tube 2), plus 0·0007 N, $CaCl_2$ (tube 3), and in tube 4 plus both NaCl and $CaCl_2$. (After G. H. A. Clowes).

Sodium soaps, used as emulsifying agents, produce oil-in-water systems (secretions ?), while the calcium and magnesium soaps favour the water-in-oil type. Therefore, in a mixture of these soaps there will be a competition for the surface of the oil drops. If the lime salt predominates interfacial tension will become greater, while it will be markedly decreased when a superabundance of sodium is present. This is clearly illustrated in Fig. 23. In tube (*a*) the oil is allowed to drop from a capillary tube (stalagmometer, Part II.) into very dilute sodium hydrate (N/1,000). This may be taken as the standard = 5 drops per unit time. The second tube demonstrates unequivocally what happens when, under the same standard conditions, sodium chloride (N/6) is added to the soda. There are now 30 drops per unit time—the surface tension has been lowered 3,650 times. The substitution of the chloride of calcium for that of the monovalent sodium (N/1,000) cuts down the drops

to 3 per unit time. That is, surface area has been decreased and surface tension increased. The final tube indicates graphically that a balance may be struck between the mono- and the divalent cations, producing a result differing not one whit from that shown in the first tube where both chlorides were absent. In our later studies we shall see in just how many reactions monovalent cations like sodium, potassium and guanidine are antagonised by divalent cations like calcium and magnesium.

The behaviour of sodium and calcium soaps in emulsion-making throws light on some peculiar problems in physiology. Loeb and his co-workers found that certain marine organisms died when put into fresh water. This will not appear surprising to the student who remembers the phenomena of endosmosis, *e.g.*, plasmolysis, haemolysis, etc. That this explanation is not correct is shown by putting the organisms into solutions of sodium chloride or of calcium chloride having the same osmotic pressure as sea water. If, however, the organisms which would have been killed by immersion in these isotonic solutions were placed in a solution having a definite ratio between the amount of sodium and calcium present, life was maintained quite normally. All protoplasm may be considered as an emulsion of lipoid material in a colloidal-crystalloidal complex. The presence of the sodium soap formed by interaction with the lipoids causes the formation of a lipoid-in-water emulsion, while the calcium soaps emulsify water-in-lipoid. The two types of emulsion thus formed are in equilibrium with an environment containing a definite Na/Ca ratio, that of sea water. Alteration in this ratio upsets the balance between the two types of emulsion and causes the cessation of growth and subsequently of life (see Nerve, Chap. XVIII.).

Soap, above certain concentrations, exists as *neutral* undissociated colloidal matter with a certain amount of water of hydration " bound " in it. If the concentration of the soap is decreased, some of it will become ionised, and so cause the " free " water of solvation to give an *alkaline* reaction to litmus. Heating a neat soap causes it to liquefy, *i.e.*, to form *liquid crystals.* Maclennan (1923) carried out work on the microscopic structure of soaps, much of which is of interest to physiologists. He showed that liquid neat soap had some kind of molecular structure or orientation indicated by its power of rotating the plane of polarised light (*q.v.*), *i.e.*, the liquid is anisotropic like a solid crystal. The crystal structure also produces characteristic X-ray photographs.

Soap solutions may be broken up in various ways.

(*a*) The addition of an acid stronger than the fatty acid frees the fatty acids, *e.g.* $H_2SO_4 + 2NaA = Na_2SO_4 + 2HA$.

(*b*) On adding a powdered neutral salt to a soap solution the soap is "salted out" as a curdy mass. The salt reduces the hydrophilic powers of the soap and so reduces the stability of the dispersoid. This is a different phenomenon from the precipitation of a colloid by electrolytes.

(*c*) On adding a soluble salt of calcium or magnesium to a soap of ammonium, sodium or potassium a curdy precipitate is produced. This curd is a calcium or magnesium soap, which, as we have seen, has little or no affinity for water.

(*d*) Solvents of soaps added to a water-soap dispersoid lead to a partition of the soap between solvent and dispersion medium. The effect of the **anæsthetics** on soap sols is interesting. *Alcohol* brings about a rapid separation of soap and water, practically all the soap dissolving in the alcohol. *Chloroform* has much the same effect, but the partition is not so complete. To get anything like a complete extraction large amounts of chloroform must be used. *Ether* has hardly any effect.

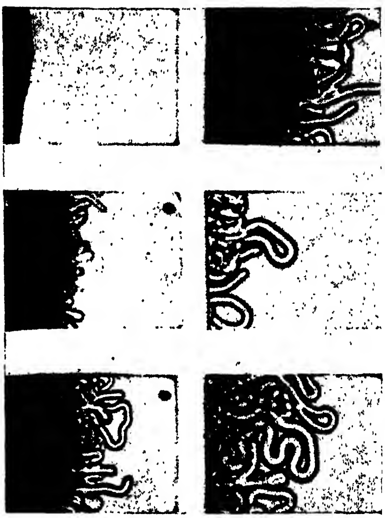


FIG. 24. Successive phases in the development of myelin outgrowths from a streak of lecithin in n/100 hydrochloric acid. (Courtesy of Professor Leathes.)

Soaps have a powerful effect in lowering surface tension, which effect is greatly increased by the addition of small quantities of alkali (Shorter and Ellingworth). A stalagmometer reading of oil dropping into water was 65 drops. When 1 per cent. soap was added to the water the drops increased to 260 (Hatschek).

Myelin Forms.—If a drop of a soap of an unsaturated fatty acid is allowed to come in contact with a drop of water, the soap will be partially dissociated into base and fatty acid, and so cause a rearrangement of the internal structure of the fat droplet. The visible sign of this alteration is the shooting out of a knob of material into the water. Close examination of this extrusion reveals that it is a coiled structure (Fig. 26 (*a*)) with a distinct adsorption membrane at the water-soap interfaces. If a micropolarimeter is used the coils will appear brightly coloured and marked with a cross which rotates with the Nicol prism. One associates this appearance with the formation of acicular crystals, and, under the high power, bipyramidal crystals may be seen lying

parallel to one another and at right angles to the myelin sheath (Fig. 26 (*f*)). If now the surrounding water be made slightly alkaline, say, altered from *p*H 7 to 7·4, the coil will steadily unroll (Fig. 26 (*b*)) till it assumes the appearance in Fig. 26 (*c*)). Fig. 26 (*d*) indicates that it is still a double structure. This stretching or unrolling, due in the first instance to increased alkalinity on the surface of water and soap, is carried out by the increased internal tension developed in the myelinated soap by the imbibition of water. The addition of a quantity of a weak acid just sufficient to ensure that the water is merely acid (*e.g.* *p*H 6·9) will cause the processes to retract (Fig. 26 (*e*)). If a greater acidity is developed the sheath will be ruptured and the crystals disseminated and dissolved. Leathes has demonstrated that lecithin and cholesterol oleate, compounds very widely distributed in the body, readily show the development of myelin forms. (See also Chap. XI., Membranes.)

FIG. 25.—Myelin outgrowths from lecithin after 24 hours in equal parts of n/100 calcium and sodium hydroxides. (Courtesy of Professor Leathes.)

Bragg and others have shown that by means of X-rays, diffraction patterns of crystals can be obtained. For instance, a single

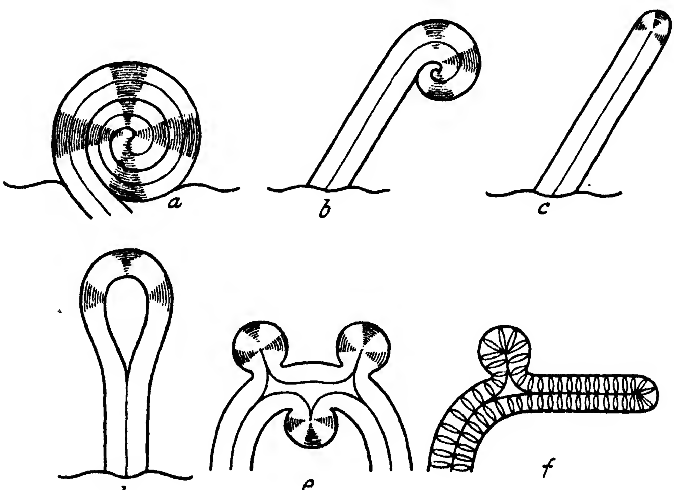


FIG. 26.—Myelin forms of Ammonium Oleate, viewed in convergent polarised light. See text. After J. H. Clark, *American Journal of Physiology*.

solid crystal gives a regular interference pattern of sharply defined spots round a central image. If, now, we have a large number of small crystals regularly arranged (as in (*f*) Fig. 26), the diffraction

pattern from the parallel planes in the microcrystals, having one direction in common, will appear as concentric rings. The distance between the reflecting planes is twice the length of the molecules composing the crystals, so that if the length of the fatty acid chain in a soap is increased, the parallel reflecting planes in the crystals produced when the myelin form contracts will diverge. The amount of this divergence is just over 0·1 $\mu\mu$ for every additional carbon atom added.

When the soap contracts, that is, when the liquid crystals within the myelin sheath are converted into true crystals and so become more closely packed together, heat is evolved. This heat is, of course, absorbed during the converse process. The former is an example of an exothermic, the latter of an endothermic reaction.

As mentioned above, under the influence of the alkali set free by the ionisation of the soap in contact with water, imbibition is induced. Water passes in through the myelin membrane and is incorporated in the soap, just as water is imbibed by gelatin and *laminaria*. This water-in-soap colloid is neutral or faintly acid to phenolphthalein in contrast to a soap-in-water sol, which is intensely alkaline.

The rigidity of tissues is to a large extent due to their emulsion character. We have up till now considered protoplasm as a liquid, arguing that it is so because it shows the phenomena of surface tension, because it allows the ready diffusion of crystalloids into and through it, and because it reacts chemically as a liquid. On the other hand, tissues, as we handle them, are more or less rigid, having elasticity and definiteness of form. Do Pickering's solid emulsions and the Na/Ca ratio not suggest a fairly plausible explanation of this double nature of protoplasm? A cell is a water-in-protein complex, while its secretion (of similar composition) is of the protein-in-water type. The "softening" of tissues observed in various pathological states may be due to the breaking of the protoplasm-emulsion from any cause (Part II.).

Our food materials as well as our tissues are colloidal complexes. They are derived in part from the animal, in part from the vegetable kingdoms.

A. Animal foods may be classified as:

(1) Milk and its products—cream, butter, and cheese.
(2) Flesh.
(3) Eggs.

(1) **Milk** is a fine emulsion of fat in a protein-colloidal solution.

(*a*) The fat globules each seem to be enveloped by a covering of adsorbed protein.

(*b*) The chief protein in milk is caseinogen, a phospho-protein

which exists in milk as a soluble calcium compound. This compound is broken by the action of acid, and protein separates as a curd.

(*c*) The carbohydrate of milk, lactose, is split by various micro-organisms, forming lactic acid, thus souring the milk and causing curdling.

Butter is simply the fat of the milk more or less completely separated from the other constituents and forming a water-in-oil emulsion. Whole, unchanged milk shows no tendency to form butter. To form butter the fat particles are concentrated at the surface by centrifugal action (or merely by allowing the cream to rise), and then by causing the cream to sour, the fat is freed from its emulsion with the colloidal matter. Since the hydrated colloids tend to collect in the surface layer between the fat globules and the dispersant aqueous phase of the cream, churning is performed to break these layers and hasten the coalescence of the fat. "The combined efforts therefore bring about a progressive increase in the concentration of the oil with a decrease in the concentration of the hydrated colloid until the instability of the oil in hydrated colloid becomes so great as to 'break' and yield the hydrated colloid-in-fat emulsion which we call butter" (Fischer and Hooker). That milk and cream are oil-in-water emulsions can be proved microscopically. They wet paper and are not greasy to the touch. Butter is a water-in-oil emulsion, feels greasy, oils paper, and microscopically appears as a finely divided aqueous colloid phase in a continuous oil phase.

(2) **Flesh.** Under this head is included, not only the muscles of various animals, but such cellular organs as the liver, kidneys, thymus, etc. The colloidal nature of such tissues has already been dealt with (see effect of cooking, below).

(3) **Eggs.** The white of eggs is practically an albumin hydrosol containing some crystalloids, while the yolk is an emulsion of lipins (lecithin, etc.), in a hydrosol of protein (ordinary proteins. and vitellin, a phospho-protein).

B. Vegetable Foods.

In the food of man, vegetable foods play as important a part as animal products. Generally, their make up is that of a mixed hydrogel of protein, higher carbohydrates (and in the case of oatmeal, maize, nuts, certain legumes and vegetables), a fair proportion of fat. This gel is enclosed in a capsule of cellulose—a higher carbohydrate which is very resistant to the action of the human digestive juices. The capsule must be destroyed by previous treatment, *e.g.*, milling, cooking, chewing, etc., before

the contents can be utilised. Far and away the most important of our foodstuffs are derived from **cereals.** From 30 to 50 per cent. of the energy of an ordinary diet comes from them. They are generally used as flour, baked into bread, or as meal made into porridge. Wheat flour is a complex gel powder consisting of about 10 per cent. protein, about 75 per cent. carbohydrate (starch and cellulose), and about 2 per cent. fat in the colloidal state. The individual particles contain molecularly dispersed salts, sugar, water, and adsorbed gases such as air and carbon dioxide. Of the 10 per cent. of protein, gliadin forms about 4 per cent. and glutelin about 4 per cent. There is less than 1 per cent. of globulin (0·6 per cent.) and albumin (0·3) present. The mixture of glutelin and gliadin is known as gluten. Gluten is insoluble in water or in dilute salt solutions, and therefore readily forms a disperse system with water called dough. Dough is a polydispersoid composed of the glutelin (and other proteins) carbohydrates and crystalloids mentioned above, bound together by colloidal gliadin. It is a viscous semi-liquid mass which, however, may be cut like a solid, and when torn exhibits a fibrous surface. The elastic properties of dough depend upon the proportion of electrolytes present, especially on the phosphates. When it is dried it changes into a gel and later becomes brittle like glue. There is doubtless a close connection between the viscosity of flour-water mixtures, and the stickiness, rising property, power of absorbing CO_2 of the dough, hydration of the starch and the porosity and volume of the resultant loaf.

The viscosity is found to increase with the concentration of the flour and also to become greater for some time after mixing. This is doubtless due to the slow swelling of the starch and albumin. If concentrated solutions are suddenly diluted the viscosity is too great at first, but gradually approaches a normal value. This is probably caused by a slow increase in the dispersion, because when the larger particles are removed by means of filter paper normal results are obtained.

Cooking. While many reactions occur in cooking, the changes that are of paramount importance are of a colloidal nature. Dough, for instance, undergoes a marked alteration in its physical characters during the baking process. The proteins are coagulated (gel formation) and the degree of dispersion of the starch is increased. Adsorbed gases are set free and the bread "rises." Further alterations take place in the loaf after it is removed from the oven.

The physical nature of flesh is profoundly altered by subjection to cooking. In roasting, grilling, boiling, or frying, the meat is

exposed directly to heat. The proteins in the outer layers are immediately coagulated, thus forming a more or less impermeable covering which prevents the escape of the meat juices, leaving the centre portion of the flesh only slightly altered chemically, but with all sols converted into hydrogels. On the other hand, if the meat is immersed in cold water and boiled, much of the protein-sol and practically all the salts and extractives are dissolved out and form soup. In this soup the protein-sol is coagulated as the temperature rises, and on cooling it is adsorbed to the surface and often is removed with the fats as a scum. The remaining meat undergoes coagulation, but is flavourless. Stewing is a modification of boiling, but the extractives, salts and soluble proteins, are served as gravy.

Further Reading

Fischer and Hooker. "Fats and Fatty Degeneration." Messrs. J. Wiley & Sons.

CHAPTER X

ENZYMES

THE TOOLS OF THE CELL

"*Instances of Magic*; . . . . By which I mean those wherein the material or efficient cause is scanty and small as compared with the work or effect produced; so that even when they are common, they seem like miracles, some at first sight, others even after attentive consideration." BACON.

THE living cell is a factory where, without any great display of energy, work is carried on which, outside the body, could only be done by the use of strenuous processes. In the cell are prepared secretions which act on insoluble raw material, rendering it soluble and so fit for transit to the cell and passage into it. Within the cell, these prepared materials undergo further change; some are used as sources of energy; from others, the cell builds up complex tissue; others again are altered somewhat and stored for future use. The cell manufactures from the material supplied, various substances, such as are required, it may be, by distant cells which are so occupied by some special process that they are unable to perform the particular synthesis. The by-products of manufacture are rendered harmless by processes possible, as yet, only in the cell. Some cells, as indicated above, have a specialised function. To a certain extent, all the cells of a multicellular organism are specialised. They are divided into communities, each engaged on some special work and requiring special raw material. Some of these communities, however, engage to a certain extent in general manufacture. They are almost, though not quite, self-supporting. The white cells of blood, for instance, are really unicellular organisms. Other communities are almost entirely dependent on imports for their sustenance. Nerve cells, for example, form the means for intercommunication between cell-communities. Their general metabolism is peculiar.

Contrast the quiet, economical, and neat living-factories with the places where things are made outside the body. Our manufacturing cities are not spotless nor are our processes there economical. Smoke, sound, and slag-heaps are universal accompaniments of a manufacturing community. Most of the processes carried on in the cell have not been reproduced in the laboratory.

Fischer, the finest physiological chemist of this or any century, has failed to synthesise the simplest protein. Fat and carbohydrates are interconvertible *in vivo* but not *in vitro*. True, steps have been taken towards the building up of a protein. Polypeptides—compounds containing eighteen amino acids—have been the crown of Fischer's efforts, but at what a cost of material, time, and energy. It has been well said that laboratory processes are just a roundabout way to the sink.

How does nature accomplish her work ? What tools does she use ? How does she harness her power ?

Nature employs catalytic methods. A catalyst is defined as a substance which, while not entering into the final product of the reaction, alters its rate and in some cases alters the point of equilibrium. A model may make this clearer. A sheet of glass may be inclined at such an angle that a body placed at its upper end just slips slowly to the foot. The momentum of the sliding body may be insufficient to carry it to the foot of the glass plate, and motion may thus stop midway down the plane. If a small quantity of oil be placed either on the glass or on the bottom of the weight, it will slide *rapidly* to the foot of the plane. The oil remains unchanged. No energy has passed from the oil to the weight, and yet the rate of falling and the point of equilibrium have been altered. The lubricant may be taken as representing a catalyst. Some one has said that a catalyst, like a tip to a waiter, accelerates a reaction that otherwise would proceed with infinite slowness. It takes no part in the main reaction, is adsorbed to the reacting body, and may be recovered intact at the end of the reaction by destruction of the substrate.

Catalysts are of very many kinds, and the mechanism of their action is so varied and so little understood that few, if any, general principles can be enunciated. They may be classified according to the means they adopt to influence a reaction.

1. **Contact agents.** Many reactions seem to be accelerated by the adsorption of the reacting substance on the surface of the catalyst, *e.g.* colloidal catalysts.

Colloids, as we have seen, are characterised by the development of surface. If we take a sphere of metal which just fits into a cubical box, and divide that sphere into smaller spheres of uniform size, the same mass of metal may be packed into the box regardless of the size of the spheres, provided they are uniform in size. Mass and total effective volume are not altered, but surface is increased. The surface of a sphere is $4\pi r^2$. If the original sphere be divided into 100 small shot, then the new surface would be $100 \times 4\pi r_1^2$ where r_1 = radius of small shot. Now $r_1 = r\sqrt[3]{\frac{1}{100}}$, so that the

ratio of the new surface a_1 to the original surface a would be

$$\frac{4\pi r^2 \left(\sqrt[3]{\frac{1}{100}}\right)^2 \times 100}{4\pi r^2} = 4{\cdot}64,$$

i.e. the surface would be increased over four and a half times. If the subdivision were carried still further till there were 10^{30} small shot, then the total adsorbing surface would be increased 10,000,000,000 times. The intensity of adsorption is chiefly dependent on the area of adsorbing surface (cf. Table IX.). In other words, contact catalysis is indicated where the specific surface of the catalyst comes within the colloidal range. Charcoal is used as an adsorbent in the clarification of sugar. A cubic metre of charcoal consisting of particles 1 mm. in diameter has a surface of about 600 sq. metres. If the particles are reduced to colloidal dimensions, say to $0{\cdot}1\mu$ diameter, then the adsorbing surface becomes 60,000,000 sq. metres. Capillary active substances, *e.g.* anæsthetics, by being themselves adsorbed to the surface of the catalyst prevent contact catalysis.

2. **Carriers.** In some cases the catalytic agent combines chemically with one of the reacting substances to form an unstable intermediate compound. This, in turn, breaks up, regenerates the catalyst, and liberates the reagent in the active atomic state—so called *nascent*. Many oxidations and reductions are brought about in this way. That is, if a reaction of the type

$$A + B \rightarrow AB$$

takes place very slowly under ordinary conditions, a catalyst C which interacts with A (*e.g.*), thus $A + C = AC$, and AC itself is acted on by B,

$$B + AC = AB + C$$

may materially alter the rate at which the whole reaction proceeds.

3. **Ionic Catalysts.** Hydrogen and hydroxyl ions act as catalysts for many reactions which occur in aqueous solution. The velocity of such a reaction in dilute solution is proportional to the concentration of the ions in question, provided the thermodynamic environment remains constant. The ion probably acts as a carrier, forming an unstable perhydrate as intermediate product.

The following statements are a brief survey of the characteristics of catalysts :

(*a*) A very small amount of catalyst can produce a considerable alteration in the rate of reaction.

(*b*) No amount of catalyst can start a reaction that would not otherwise take place.

(*c*) A catalysed reaction reaches the same final state as ultimately it would reach if no catalyst were present.

(*d*) Catalytic acceleration (positive or negative) is proportional to the concentration of the catalyst. This is true only within limits. At very high concentrations of catalyst the acceleration is not quite proportional to concentration.

(*e*) The catalyst is not destroyed during the reaction, but may suffer a change in physical state or be altered chemically by some subsidiary reaction.

(*f*) Some catalysts are specific in their action. They act best in certain reactions. For example, hydriodic acid is slowly oxidised by hydrogen peroxide and by persulphates. The former reaction is activated by tungstic acid, but not the latter.

The great majority of vital catalytic reactions have, as catalyst, an enzyme. Enzymes themselves cannot be detected or estimated. Their presence is made apparent by their action. By estimating the amount of the products of enzyme activity an idea of the rate of reaction may be gained. Many attempts have been made to isolate and purify certain enzymes and, though complete success has not been granted to any investigator, much has been learned of their nature and of the conditions necessary for enzyme action.

(*a*) **Enzymes are colloidal.** They can readily be separated from crystalloids by dialysis or ultra-filtration. Chemically, they resemble their substrate or are so closely associated with their substrate that existence apart is impossible. It may be that the colloidal character of enzymes is the secret of their action. At any rate, an artificial oxidising enzyme has been prepared by mixing a suspensoid—finely divided manganese, with an emulsoid—gum acacia. The adsorption complex so formed, if suitable crystalloids were present, reacted as an artificial "laccase."

(*b*) Enzymes retain their activity only over a very well-defined range of **temperature.** It is common knowledge that physiological processes take place most rapidly at body temperature. Every biological laboratory is equipped with devices for keeping incubators at a constant temperature—say, 37°–40° C. Before these appliances had been perfected, investigators in this realm had to keep their experimental material on their person. The Abbé Spallanzani (1729–1799), in his classical work on digestion, carried his digest-tubes in small pockets in his armpits for several days. During the Great War, when scientific work had to be carried out in all sorts of places, at least one physiologist, bereft of gas regulators, had to resort to this simple but efficient method of maintaining a fairly uniform temperature. In this way, reactions in which they play a part differ from those usually styled chemical. The rate of most chemical processes is doubled or trebled when the temperature is raised 10° C. The enzymes

follow this rule only from 0° C. to a temperature called their *optimum temperature*, above which the rate decreases rapidly. The optimum temperature of most enzymes lies between 30° and 40° C. The decrease in rate of reaction when the temperature is allowed to go over 40° C. is probably due to coagulation of the enzyme. Increase in temperature causes alterations in the physical state of colloidal matter. These alterations, in viscosity, in colour, and in conductivity, all indicate an increase in the size of the colloidal particles, and consequently a decrease in their specific surface. The effective adsorbing surface is diminished. At the optimum temperature the increased chemical action due

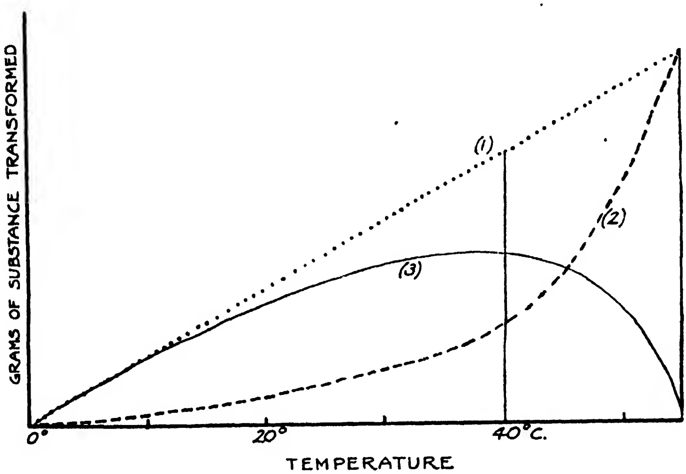


FIG. 27. - Graph to show how the effect of increase of temperature on the rate of enzyme action is the result of the interaction of two factors, (1) increased chemical action and (2) increased destruction of enzyme.

to temperature more than balances the decreased adsorbing surface. Beyond this temperature, the loss of surface becomes relatively important. If the temperature is raised till the specific surface is reduced, by coagulation, to a value below 10,000, adsorbing power is totally lost, chemical action is stopped, and the enzyme is said to be dead.

In the appended figure (Fig. 27) curve 1 (dotted line) shows how, as the temperature increases, a pure chemical action is accelerated. Curve 2 (dash line) represents the rate at which the effective surface is decreased by rising temperature. The process, it will be noticed, is not an instantaneous one, but proceeds with a definite velocity which increases very markedly somewhere about 30° C. Curve 3 (firm line) is the graph of the rate of the same chemical reaction as shown in (1), but carried out by enzyme

action. This curve may be drawn by plotting the differences of the ordinates of (1) and (2) on the same scale of temperatures.

(*c*) The **hydrogen ion concentration** of the medium in which the enzyme acts has much to do with its activity. Each enzyme is active only when the bathing fluid has a p_H of a certain range with an optimum p_H at which the action proceeds at its best. The extraordinary sensitiveness of colloids to the p_H has been mentioned.

(*d*) The **crystalloid** content of the substrate solution is peculiar for each enzyme. Certain salts are, of course, destructive. All salts which break up colloidal complexes, inhibit or destroy enzyme action. Enzymes are " salted out " by the neutral salts that precipitate colloids and may thus be separated.

(*e*) **Anæsthetics** have no effect on enzyme action.

Chloroform, thymol, etc., may therefore be used to keep experimental enzyme solutions free from bacteria.

To sum up,—the ranges of temperature, p_H, salt content, etc., all point to the colloidal nature of enzymes.

The material on which an enzyme acts is called its **substrate**, and each enzyme acts on a specific substrate and on no other. In many cases the name applied to the enzyme is derived from that of its substrate by altering the terminal syllable to—ase. Thus maltase acts on maltose.

Lactase	acts on	lactose
proteinase / protease	,,	protein
aldehydase	,,	aldehyde
lipase	,,	lipides
peroxidase	,,	peroxide
arginase	,,	arginine
urease	,,	urea.

Sometimes the function of the enzyme may be indicated by its name, viz. :

oxidase	accelerates	oxidation (= peroxide + peroxidase)
catalase	,,	breaking down of peroxides
invertase	,,	inversion of cane sugar
desamidase	,,	removal of amino groups.

The majority of enzymes of physiological importance, however, have no accepted systematic name. They are the ones first known and they were named to suit the fancy of their discoverer. Ptyalin (Gr. Pteuin—to spit) acts on starch and should be called salivary amylase. Several others are in a similar position, *e.g.* Pepsin (Gr. Pepsis—digestion) = acid or gastric proteinase.

Trypsin (Gr. Tribein—to rub—prepared by rubbing pancreas with sand and glycerol) = alkaline or pancreatic proteinase.

Some writers prefer to use names which point to the splitting power of the enzymes, *e.g.*

proteolytic or proteoclastic enzymes	act	on	proteins
amylolytic or amyloclastic	,,	,,	starches
lipolytic or lipoclastic	,,	,,	fats.

On the other hand, hydrolytic enzymes produce their effect by adding or subtracting water.

Some enzymes act *in* the cells while others are secreted by the cells and act on a substrate *outside* the cell. The former, **endo-enzymes**, have been little studied. An active suspension of them may be prepared by grinding up tissue with sand and extracting with watery glycerol. It is probable that all muscle cells contain enzymes which act on protein-disintegration products, either rebuilding proteins from amino acids or breaking down these amino acids. Similarly, the regeneration and the disintegration of carbohydrates and fats have been attributed to endo-enzymes. There are also special enzymes to carry out oxidations and reductions in the cell. The various stages in the production of uric acid from nucleoprotein have been studied exhaustively, and each stage has been shown to have its enzyme or series of enzymes.

The **ecto-enzymes** are secreted in the various digestive juices and act on their substrates in some portion of the alimentary canal. They really act outside the body and have one function only—to break down the food into a state in which it can pass through the gut wall into the body.

Secretion. Some of these enzymes seem to be secreted ready for action. They themselves are in the active state, and the juice of which they form a part contains the necessary salts and has a suitable P_H. The moment that the juice comes in contact with the substrate, digestion begins.

Zymogen Secretion. Others, however, enter the alimentary canal in an inactive state. Their inactivity is not due to the lack of a suitable medium, but to the form in which the enzyme appears, *i.e.* as a pro-enzyme or precursor of the enzyme. An activator is required, **pseudo-activation.** The active principle of gastric juice is secreted as pepsinogen, which becomes active pepsin on coming into contact with a fluid of a certain P_H. This is not a true activation. Acid does not so much activate pepsinogen as form a necessary concomitant for pepsin. That this is so may be demonstrated by neutralisation of the acid, with consequent loss of activity in the enzyme. On reacidifying, digestive activity

restarts. Acid and pepsin have been termed co-enzymes—a misleading term. **True activation** is irreversible. Once an enzyme has been rendered active its activity cannot be withdrawn or restored at will. As an example of true activation, the pancreatic enzyme trypsin may be taken. Pancreatic juice drawn from the duct contains trypsinogen. This precursor gives birth to active trypsin on coming into contact with *enterokinase* of the succus entericus. The mechanism of the change is unknown. Enterokinase is an enzyme whose sole function is to act on the zymogen form of trypsin. No other protease can be substituted. The rate of activation is peculiar and suggests autocatalysis—*i.e.* it starts slowly at first and the rate rapidly increases with time. Vernon suggests that a third enzyme, deuterase, acts as a middleman.

A simpler explanation might be found in the adjustment of equilibrium between two hydrophilic colloids with different crystalloid contents.

In order to explain the immunity from digestion of the living cells, **anti-enzymes** have been postulated. The stomach wall, for instance, contains protein which is not digested by gastric protease as long as the blood supply is intact. Occlusion of the blood supply to any part leads to the formation of a gastric ulcer. Parasitic worms live in contact with enzymes that would cause rapid digestion in the event of their death. Neither Cohnheim nor Bayliss is inclined to accept the anti-enzyme idea as correct. (1) The latter has shown that the phenomenon can be explained without any such hypothesis—*e.g.* by the adsorption of the enzyme by another colloid. Agitation of a suspension of trypsin with charcoal results in a loss of digestive activity due to the adsorption of the enzyme by the charcoal. The charcoal here acts as an anti-enzyme. (2) Enzymes as colloids are sensitive to any alteration in their environment. A slight alteration in salt content, colloid or water concentration, or P_H leads to alteration in their power of adsorbing or being adsorbed by their substrate.

We are now in a position to consider if enzymes should be admitted as catalysts. Do they have the six characteristics detailed on p. 117 ?

(*a*) *Minute Quantities.* They undoubtedly accelerate reactions when present in amounts even more minute than an inorganic catalyst, *e.g.* in the reaction

$$\text{Lactose} + \text{water} = \text{glucose} + \text{galactose}$$

one might employ as catalyst either an enzyme or an acid. The enzyme *lactase* is about 5,000 times as effective as an equal weight

of hydrochloric acid. Pepsin can hydrolyse about 400,000 times its weight of caseinogen.

(*b*) *Do not Initiate Reactions.* This is a much more difficult canon to satisfy. Certainly cane sugar *in solution* slowly undergoes inversion, but who is to say whether it would invert in the process of time if kept dry. The process might be proceeding at an immeasurable rate.

(*c*) *Final State of Equilibrium.* Enzymes do appear to change the equilibrium point of reversible reactions in some cases, *e.g.*

No Catalyst. Starch + H_2O → dextrin + maltose + glucose
Catalyst-HCl. ,, ,, → glucose
,, Amylase ,, ,, → maltose.

i.e. the reaction takes place in steps. In the presence of water all the stages are shown, while when acid is added only the final product appears in quantity. Various enzymes activate the various steps—one enzyme aids in the production of dextrins from soluble starch, another assists in the process of hydrolysing the dextrins to maltose, while still another catalyses the step down from maltose to glucose. Each of those steps should be considered a separate reaction.

(*d*) *Activation Proportional to Concentration.* This is strictly true of enzymes, and may be proved in various ways (Part II.).

(*e*) *Not Destroyed.* That enzymes are altered during a reaction is not surprising considering their unstable colloidal nature. The change entailed is usually an alteration in physical state whereby they are rendered inactive.

(*f*) *Specificity.* Each enzyme acts on a specific substrate, and if the substrate is a mixture of optical isomers, one of these (and always the same one) will be selected for preferential treatment. Examples may make this clearer. If maltase be added *under suitable conditions* to the following disaccharides it will be found to act preferentially on one—maltose.

TABLE XX

Sugar.	Components.	Split by.
Maltose . .	Glucose α glucoside . .	Maltase.
Isomaltose .	Glucose β glucoside . .	Emulsin.
Centiobiose .	Glucose β glucoside . .	Emulsin.
Cellobiose .	Glucose β glucoside . .	Emulsin.
Lactose . .	Glucose β galactoside .	Lactase, (crude emulsin).

TABLE XX—*continued*

Sugar.	Components.	Split by.
Isolactose .	Glucose galactoside . .	?
Melibiose .	Glucose galactoside . .	Melibiase (crude emulsin).
Trehalose .	Glucose + glucose . .	Trehalase.
Cane Sugar .	Glucose + fructose . .	Invertase.
Turanose .	Glucose + fructose . .	? (not invertase).

Lactose and melibiose are both glucose galactosides differing only in the position occupied by the hydroxyl of the glucose molecule united to the galactoside. As galactosides, both are slowly hydrolysed by crude emulsin (known to be a mixture of at least three enzymes). Lactase is, however, without action on melibiose, and melibiase does not split milk sugar. Till further experimental work has been done attempted explanation of these facts is mere guesswork. Fischer has suggested that the enzyme is to its substrate as a key is to its own particular lock. The evidence at present available does not altogether lend itself to this explanation. It looks as if a careful study of the alterations brought about in the configuration of colloids by slight modifications of the surrounding conditions might lead towards an acceptable explanation of specificity. (See also Optical Activity, p. 126.) Weight is given to this suggestion by examination of the **synthesising power of enzymes.** Since enzymes accelerate reactions that would take place without them, and since, theoretically, all reactions are reversible, the synthesis of complex bodies from their constituents might be expected by the aid of the same enzyme as brought about the splitting of the complex. That is, a lipase should not only split a fat into fatty acid + glycerol, but should regenerate fat from fatty acid + glycerol.

A reversible or balanced reaction is one in which, under definite conditions, there is a certain equilibrium point at which the amount of material being broken down is exactly balanced by the amount being built up. For example, take a stoppered bottle half full of water. Two processes are going on simultaneously. (*a*) Liquid water is undergoing vaporisation and the gaseous hydrol is passing into the air, (*b*) Gaseous water particles are passing from the air into the water to form, say, dihydrol. When the air is saturated with humidity for that particular temperature, exactly the same number of water molecules will leave the water

as enter it. Now alter the conditions, (1) open the bottle to a dry atmosphere, *i.e.* to unlimited air containing infinitely little moisture. The reaction will proceed almost entirely in one direction—evaporation. (2) If the bottle be opened to an air supersaturated with moisture, the reverse process, condensation, will predominate.

The effect of the removal of the maltose in increasing the speed of digestion of starch is shown very clearly in the following experiment in which the course of the digestion of the starch was followed by the iodine reaction. In one case, the digestion was carried on in a beaker, and in the other in a dialysing tube immersed in running water so that the maltose dialysed out.

TABLE XXI

Time (in mins.).	Dialysed Starch Solution.	Not Dialysed.
0	Pure blue	Pure blue
20	Trace of violet	,, ,,
40	Red with violet tinge	Violet
75	Colourless	Red with violet
100	,,	Very faint red
125	,,	Colourless

Ecto-enzymes generally have a catabolic function. The by-products of their activity are removed as rapidly as they are formed. Many endo-enzymes have for the most part an anabolic activity. They are brought into contact with simple compounds and proceed to build them into more complex substances. All enzymes have both breaking-down and building-up functions. *The conditions under which they work determine their function.*

A peculiar phenomenon has been noted in this connection, namely, that the substance built up by an enzyme may not be quite the same as the complex substance originally broken down by it. Maltase, for example, splits maltose into molecules of glucose, but the substance formed by the action of maltase on glucose is not maltose but its β form, isomaltose. On the other hand, isomaltose is split by emulsin into glucose, while emulsin causes two glucose molecules to unite to form maltose. This is explained by supposing that the nature of the enzyme-substrate complex influences the rate of the reaction. If HCl is used as catalyst, the equilibrium point is reached with glucose, maltose, and isomaltose present in the same proportions, irrespective of the original proportions present in the substrate. The point of equilibrium is changed by the enzyme. If maltase is used, the proportion of maltose is diminished, but if emulsin is the enzyme

employed the point of equilibrium is shifted towards isomaltose. The whole subject requires re-examination from the point of view of colloid chemistry, especially with regard to the influence of P_H on activity. The following table gives the optimum P_H for certain enzymes :

TABLE XXII.

Ptyalin	6·7
Invertase	4·5
Maltase acting on maltose	6·6
,, ,, methyl-glucoside	6·2
Pancreatic lipase	8·0
Pepsin on proteins	1·5–2·5
,, (plastein formation)	1·0
Rennin	5·7
Trypsin on peptone	7·7
,, gelatin	9·7
Erepsin	7·8
Urease (Decomp. of urea)	8·7
,, (Synth. ,,)	7·0

Much has been made of the fact that enzymes seem to be rather finical as to what compounds they will attack. Two compounds may exist side by side similar except in one respect. They may differ in structure as the right hand differs from the left. That is, the one compound is structurally a mirror image of the other. The enzyme selects one for attention and hardly looks at the other. If the enzyme is engaged in synthesis, it invariably builds right-handed sugars and left-handed leucine (an amino acid). If engaged on demolition, the enzyme will hydrolyse all or nearly all of the right-handed sugar before touching its mirror image, and similarly with *l*-leucine. How can this be explained ?

Optical Activity.

It is obvious that a paper-cutter or strip of metal can pass through a book only in the plane of the pages, and may pass through a second book when both books are similarly placed or when one has been placed upside down, *i.e.* rotated on its central axis *AC* by 180° (Fig. 28). If, after passing through book one, the

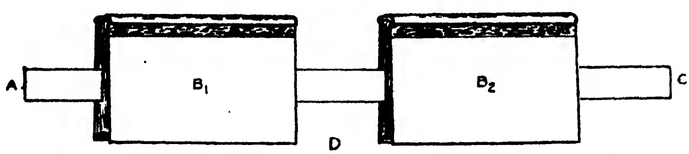


FIG. 28.—Model of polarimeter. *A*=source of light, B_1=polariser, *D*=point at which twisting force is applied, B_2 analyser, the amount of twisting at *D* can be estimated from the angle through which B_2 has to be rotated to allow of the passage of the metal strip *AC*, *C*=eyepiece.

strip of metal is given a twist, then book two will have to be turned through a corresponding angle before the metal will slip through its pages. The rotation of book two may be taken as an index of the twisting of the plane of the metal strip. Various factors may modify this twisting :

(*a*) The nature of the metal. The same twisting force would produce very different results in, say, copper and steel.

(*b*) The length of the strip exposed to the twisting force. The longer the strip between B_1 and B_2 the greater will be the twisting, other conditions being equal.

(*c*) Temperature. Increase in temperature will increase the twisting.

(*d*) Obviously the nature and strength of the distorting force will modify the angle of rotation of the strip.

A polarimeter is a device in which these basal facts are applied to light. If a beam of light (at A) is made to take the place of the metal strip and for the books we substitute some optical arrangement which will allow light vibrating in one plane only to pass, then the eye (at C) would see a lighted field when B_1 and B_2 were in the same plane, and only then. As we shall see presently, the plane in which polarised light vibrates may be twisted by the action of various crystals and of several substances in solution and when fused. The amount of the rotation depends on the factors enumerated above, viz. :

(*a*) The nature of the light. The angle of rotation depends on the wave-length of the light ; the shorter the wave length the greater the rotation.

(*b*) The length of beam exposed to the optically active material.

(*c*) Temperature as above.

(*d*) I. Nature of the optically active material : each such has a specific rotatory power.

II. Strength of solution ; double the concentration produces double the rotation.

The modification of a prism for producing light vibrating in one plane was devised by Nicol and so bears his name. He made use of a property of Iceland spar (calcium carbonate), namely, its double refraction. Iceland spar crystallises in many forms, but they are all split most readily along certain planes which are all inclined to each other at fixed angles, and by cleavage the crystals can always be reduced to the rhombohedral form. If such a crystal of Iceland spar be placed on a piece of paper in the centre of which a black dot has been made, on looking down through the crystal, two black dots will be seen. If now the crystal be rotated without lifting it from the paper, one dark spot will remain

stationary while the other will rotate round it as a centre. This phenomenon of double refraction may be demonstrated in another way. If a strong beam of light be allowed to fall on one of the faces of a crystal of Iceland spar and the transmitted light be received on a screen, two spots of light will be seen, and if the crystal be rotated as before, one spot will circle round the other. That is, the beam of light has been split into two rays of equal intensity.

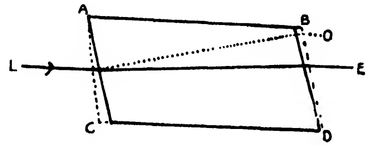


FIG. 29.—Diagram of the paths of the ordinary and extraordinary rays of light through a rhombohedron of Iceland spar. The light, falling on the face *AC*, divides into two rays, both of which are polarised. The extraordinary ray (*E*) is the lesser refracted ray: the ordinary ray (*O*) is the more refracted ray.

One ray, the stationary one, has travelled through the crystal just as it would pass through glass—obeying the ordinary laws of refraction (Snell's Law). It is called the ordinary ray. The other ray is called the extraordinary ray, and it does not obey the ordinary law of refraction. It is this ray which gives the movable image when the crystal is rotated. (Snell's Law states that the ratio of the sine of the angle of incidence to the sine of the angle of refraction is constant, $\frac{\sin \alpha}{\sin \beta} = \mu$.)

Both rays are plane polarised, but in planes at right angles to one another. Nicol's problem was to get rid of one of these rays

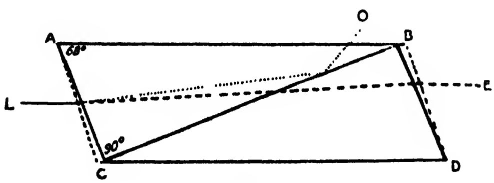


FIG. 30.—Diagram of refraction in a Nicol's prism.

so as to get light vibrating in one plane. The method he adopted is very ingenious. The angular separation between the ordinary and extraordinary rays is not very great, so that it is not possible to screen off one of the rays unless a very thick crystal be employed.

A rhomb of Iceland spar was cut in two by a plane *BC* (Fig. 30) perpendicular to the principal plane for the face *AC*. The cut surfaces were carefully polished and then cemented in their original position by a thin film of Canada balsam. If now the ordinary ray falls on the surface *BC* at an angle greater than the

critical angle it will be totally reflected, while the extraordinary ray will pass through the prism. This ray, as we have stated above, is plane polarised. To the unaided eye it differs in no way from ordinary light, but, when viewed through a second Nicol's prism, its condition is recognised by the fact that on rotating the prism the beam of light from the first prism alters in colour, passing through the various colours of the spectrum and returning again to white when the rotation has been carried through 180°. If monochromatic light has been used the field will be illuminated when the principal planes of the two prisms are parallel. On rotating the second prism through an angle of 90° the ray is extinguished and the prisms are said to be crossed. If the rotation be carried on to 180° the planes are again parallel and again the field is bright, and so on. In two positions the planes are parallel and in two at right angles. The first prism is called the *polariser*, the second, by which alone we can recognise the polarisation of the

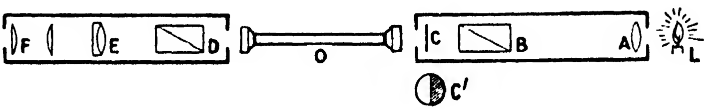


FIG. 31.—Diagram of Laurent Polarimeter. Monochromatic light from the source *L* passes through the lens *A* which renders the rays of light parallel, and then through the polariser *B*. *O* is the observation tube containing the fluid under examination, while *D* is the analysing Nicol prism. The field of view is observed through the telescope *EF*. At *C* the circular opening of the tube carrying the polarising prism is half covered by a thin quartz plate (shown at *C'*), the thickness of which is such that the light in passing through the plate is altered in phase by half a wave-length.

light from the first, is called the *analyser* (Fig. 31, D). If now a plate of quartz cut with the faces perpendicular to the optic axis be placed between *crossed* Nicols, it will be found that some light passes through the analyser. That is, the quartz has rotated the plane of the light polarised by the first prism. By rotating the analyser a position can be found when all light is stopped. The amount of rotation of the analysing Nicol is a measure of the rotation of the plane of polarised light by quartz. A body which has this property of rotating the plane of polarised light is said to be **optically active.**

Some samples of quartz rotate the plane of polarisation in a clockwise or right-handed (or +) direction, other samples have a reverse (or −) direction of rotation. (The direction is taken as from the direction in which the light is travelling, not from the analysing eye.) A dextrorotatory piece of quartz superimposed on a similar laevorotatory piece would be optically inactive. Physical examination of quartz crystals shows that *d*-crystals differ from *l*-crystals in one respect only, viz.: the position of their secondary facets. The ordinary form of a quartz crystal is a six-sided prism topped by a six-sided pyramid. The alternate

solid angles where two prism faces meet two pyramid faces is generally levelled off to form a small secondary face or facet. When the crystal is viewed with the pyramid upmost and these facets slope to the right, the specimen will rotate the plane of

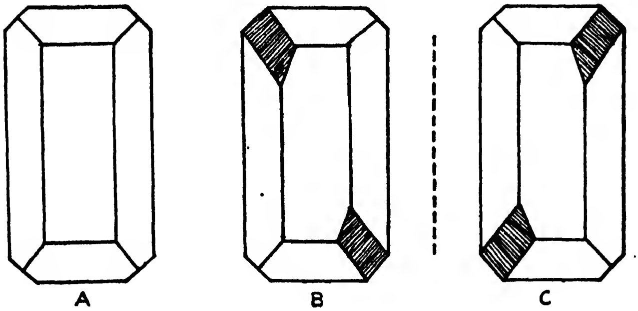


FIG. 32. Crystals of Ammonium Hydrogen Malate. (*a*) Symmetrical crystal, optically inactive; (*b*) Asymmetrical crystal, dextrorotatory; (*c*) Asymmetrical crystal, laevo-rotatory. (After van't Hoff.)

polarisation to the right, and *vice versâ* when the facets incline to the left. The one crystal is a mirror image of the other, and is called its optical isomer.

If the crystal is symmetrical with no secondary facets, then

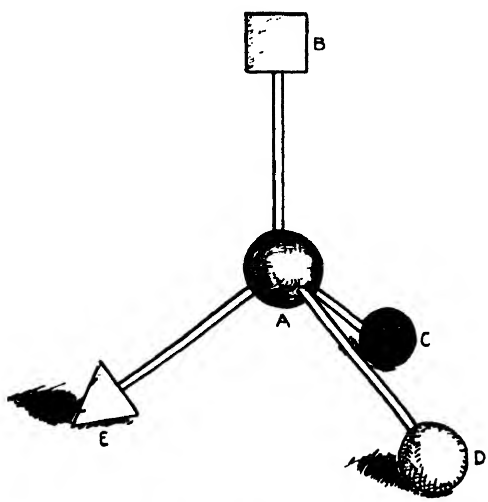


FIG. 33.—Diagram of a carbon atom (*A*) having its valencies supplied with four different atoms *B*, *C*, *D*, *E*. The mirror image of this structure would be its optical isomer.

optical activity is impossible. Any perfect cube is an exact duplicate of any other perfect cube. Such holohedral crystals can be prepared. In Fig. 32, *A* represents a holohedral crystal of inactive ammonium hydrogen malate; *B* represents a dextro-

rotatory crystal, while C represents its mirror image, a laevo-rotatory crystal of this salt.

Of amorphous bodies which are optically active, with the exception of one or two little known compounds of nitrogen, all are compounds of carbon in which one or more of the carbon atoms has its valencies satisfied by four different atoms or radicles. Such a carbon atom is termed *asymmetric* (Fig. 33). Compounds containing asymmetric carbon atoms exist in proteins, carbohydrates and fats. Each member of a pair of optical isomers is identically equal to its isomer in every respect but one.

As stated at the beginning of this section, enzymes preferentially act on one isomer. For example, those sugars which are dextro-rotatory are more readily hydrolysed than their laevorotatory isomers. The mould *penicillium glaucum* destroys *l*-leucine and *d*-glutamic acid without having any extensive action on *d*-leucine or *l*-glutamic acid.

Fischer showed that the proteoclastic enzyme, trypsin, acted asymmetrically on synthetic polypeptides, *e.g. inactive d.l.*-alanyl-leucine was digested in such a way that only the compound of *d*-alanine and *l*-leucine was hydrolysed, whereas the compound of *d*-alanine and *d*-leucine was undigested. That is, the natural isomers were destroyed by the enzyme before the isomers not occurring in nature were manifestly attacked.

Much research has been done to elucidate the reason for this preferential treatment, and some fanciful explanations have been put forward. The problem is a difficult one and the bias of the enzyme at present inexplicable. One fact, however, may be of importance for future development, viz.: if inactive reagents are used to destroy or produce compounds having an asymmetric C atom, then both isomers will be produced or destroyed equally; if, on the other hand, optically active agents are employed, one isomer has preferential treatment.

CHAPTER XI

MEMBRANES (PLASMAHAUT)

THE HOUSE OF THE CELL

"The retention of an individuality by the cell must be determined by chemical and physical differences between this layer and the surrounding fluid."

STARLING.

THE unit of life is the cell. It follows that all changes that affect life take place in the cell and that the metabolism of a complex organism is the sum of the changes of the cells that compose it. It is therefore logical to study unicellular animals with a view to the application of the knowledge so gained to the elucidation of the more intricate problems of multicellular organisms, *provided that it is borne in mind that in a complex animal, each cell will be modified in form and somewhat in function not only by neighbouring and similar units, but by comparatively remote and dissimilar cells.*

Need for cell membrane.

We have seen that the cell consists of protoplasm, which may be regarded as water dispersed through a colloidal complex containing dissociated and non-dissociated crystalloids and substances in suspension.

(i.) Now it is clear, that as amoeba, for instance, lives in water, some skin or pellicle is necessary to prevent the protoplasm from suffering infinite dilution. For example, if an amoeba is deprived of its coat, say by bringing the animalcule into contact with air at the surface of water, the naked protoplasm lying below the surface of the water after a moment or two generally suffers dispersion throughout the surrounding water.

(ii.) Further, if osmotic pressure is to be converted into hydraulic pressure, a membrane is necessary, as we have seen. In plants, growth is, in part, due to osmotic energy, and therefore plant cells must be bounded by a cell wall which will allow the passage of water, but not of, say, sugars. Nägeli, Pfeffer, De Vries and others have demonstrated the existence of such a cell wall. If plant cells are immersed in a hypertonic salt solution, *i.e.* in a salt solution having a greater osmotic pressure than the osmotic pressure of the cell contents, then exosmosis will take place. Water

will pass from the cell, causing the cell *substance* to shrink. But the cell *wall* does not shrink and a space is left between the contents and the container, so rendering the latter apparent. This process is termed **plasmolysis.** Free cells like red blood corpuscles may be submitted to experiments similar to the plasmolytic one detailed above. If corpuscles are put into a solution of lower osmotic pressure (hypotonic solution) than their contents, they will swell up because of the passage inwards of water, *i.e.* endosmosis, and will probably burst. This is called **hæmolysis,** and may be brought about in other ways, which are, however, all obviously methods for destroying a membrane (page 318). Artificial membranes may be made which act in a way similar to animal cell coverings.

(iii.) The work of A. V. Hill, Meyerhof and others, which has in recent years done so much to elucidate that process so essential for our well-being, viz. muscular contraction, has also pointed to the necessity for postulating membranes in and on the contracting units (Chap. XIV.). These experiments, together with the fact that it is quite impossible to conceive of energy changes taking place in naked protoplasm, are sufficient evidence of the need for cell membranes.

Nature of the Membrane.

The cell wall differs considerably from the cell contents in chemical composition and in physical state. In plants, it generally consists of cellulose. Certain animals develop an exoskeleton of the excreted salt of lime, of silica or of chitin. These excreted membranes should not be confounded with the true cell membrane or plasmahaut, which term connotes the layer of cell protoplasm which, in animal and plant cells alike, lies between cell and environment. Only through this layer or membrane can the cell be influenced by changes in the surrounding medium. Animal cell membranes are more elastic than those of plants. Microscopic examination shows generally a difference in refractive index round the border of cells.

Much research and much speculation has been published on the nature of these membranes. What conditions have they to fulfil ? At least four qualities are essential :

(1) The membrane must prevent the outward passage of cell substance while allowing water to pass freely in and out.

(2) The membrane must permit of the intake of nutrient material and of the output of undigested and non-utilised material.

(3) The waste products of metabolism, gaseous and liquid, must find a way out, while oxygen must find a way in.

(4) Finally, the membrane must be of such a nature as to allow

of expansion. Mere elasticity will not answer this end. The membrane must be capable of almost instantaneous growth.

The animal cell membrane must not be considered as a box or container in which the cell protoplasm has been placed. It is not something apart from the cell like an eggshell. It is not even something made by the cell and deposited outside like the crustacean shell. It is just as much part of the cell as the protoplasm itself. Unless this point is understood, difficulties will be found in the study of alterations in permeability. The animal cell membrane must be considered as a part of the cell, having a similar metabolism to the interior of the cell and dying when the rest of the cell dies. A very simple experiment shows the intimate relationship of cytoplasm and membrane. Amoebae immersed in a dilute aqueous solution of eosin remain unstained as long as they are alive. That is, the membrane is impermeable to the dye. Eosin, on the other hand, injected into the body of the amoeba remains evenly distributed throughout the protoplasm during life, and does not diffuse into the surrounding water. In both experiments the cell wall becomes permeable to the dye on the death of the animal.

Formation.

The fourth essential quality of a cell membrane mentioned above gives a clue as to the mode of its origin.

The only membrane that could answer to this test, *i.e.* as capable of instantaneous formation and expansion, is one formed by a Gibbs-Thomson deposition on the surfaces. Any substance in the protoplasm which lowers surface tension will have a greater concentration at the surface than in the interior. If there is more than one capillary active substance present, then the one in possession of the surface will be the substance having the greatest power of lowering surface tension. Now a surface implies the juxtaposition of two systems or phases, *e.g.* amoeba-water, erythrocyte-plasma, cell-environment. The second system, the environment, must, therefore, contribute its quota to the formation of the membranes. That such a membrane can be formed is readily demonstrable.

1. Brailsford Robertson's artificial amoeba (Part II., p. 516) shows mobility and keeps intact for some time.

2. Egg albumin solution forms a pellicle or coat of great toughness, cf. meringues.

3. Traube's membranes, especially in the hands of Leduc (Part II., p. 544), yield life-like growths.

Strictly speaking, a substance which is adsorbed under certain circumstances will be set free when the circumstances are reversed. Many substances, however, undergo alteration in physical state on adsorption. For example, in the formation of meringues, the egg white becomes coagulated and so becomes incapable of re-entering the liquid state. Such an irreversible reaction is termed pseudo-adsorption.

Adsorption (including pseudo-adsorption) is, as we have seen in a previous chapter, dependent on surface forces. Now, from its very nature, surface tension has a negative temperature coefficient. Increase of temperature lowers surface tension. It follows that increase of temperature will diminish the amount of material adsorbed, and, conversely, a decrease in temperature will increase the adsorbability of substances in solution. Can we associate with this fact the varying thickness of animal membranes according to their degree of exposure to cold ?

There is no doubt that material adsorbed to a surface can in turn take up other matter from the body of the solution. In fact it might completely remove one component. (Cf. emulsions and emulsifying agents.) Surface concentration alone cannot account for all the properties of the plasmahaut.

Composition of Membranes.

The exact chemical composition of animal cell membranes is not known, but modern research tends to show that it is similar to that of the cell as a whole. This is particularly true when attention is directed mainly to the lipide content of protoplasm. One is struck first of all with the way in which under physiological conditions the amount of *tissue lipide* is kept constant in all cells (except those of the liver). These unsaturated fats seem inseparable from the life of the cell. We have seen (Chap. VI.) how fats spread over a surface in a layer one molecule thick, and how slight alterations in the hydrogen ion concentration on either side of this fatty layer may make extensive alterations in the structure and intimate composition of the layer, and how fats solid at a particular temperature become apparently fluid when adsorbed.

Couple with their insolubility in water, the chemical inertness of the fats and their substitution products, and one can see how suitable they are as building stones for the house of the cell. If this view is correct, then fat solvents should cause disruption of animal membranes. This is easily shown to be the case (cf. Haemolysis by ether and by soaps, Chap. XXII.).

Cholesterol and ***lecithin*** are general constituents of cell membranes and their relative proportions play an important part in controlling

the amount of water held by the cell. The ratio of water to solids in a cell depends *directly* on the *cholesterol* and *indirectly* on the *lecithin* content. Now, Leathes has shown that if a monomolecular layer of *lecithin* is prepared on the surface of water, the area so covered is markedly reduced by the addition of *cholesterol*. In some way this alcohol causes the long-chained phospholipine to pack its molecules more closely together. Protein, cell-albumin and α globulin and a nucleoprotein (so-called β globulin) also enter into the composition of the cell membrane, and contribute to its apparently capricious behaviour by virtue of their amphoteric nature.

Structure.

In spite of many attempts to overthrow it, the most satisfactory explanation of the structure of a cell membrane is the pore theory. The question as to whether the pores are like those of a sponge or like those of a honeycomb is not of importance, for the membrane is of extreme thinness. It has been proved that the rate of passage of a fluid through an artificial membrane is the same as the rate of flow through capillary tubes. For our purpose, then, we may consider that cell membranes are composed of some of the cell material concentrated at the surface and admitting water, etc., through the spaces between the molecules or other complexes which compose this layer.

Permeability.

Artificial membranes may be prepared of any desired permeability (Part II.). A membrane which allows water to pass through and no solute is said to be **semipermeable**. A perfect semipermeable membrane has never been prepared, though Traube's copper ferrocyanide membranes are very nearly so.

If an animal membrane, such as a pig's bladder, be stretched across the end of a cylindrical tube so as to form a drum-head, one has a simple dialysing membrane such as was employed by Graham in his classical researches. When this membrane-covered end is immersed in water, the liquid cannot rush into the dialysing vessel all at once, but slowly oozes through. A solution of sodium chloride passes in almost as rapidly as water alone. Sugar passes through the membrane slowly, while a starch solution fails to penetrate at all.

A list of hydrated ions could be drawn up in the order of their magnitude or, which comes to the same thing, in the order of their speed of migration. With certain apparent exceptions, which will be mentioned immediately, the ability to pass through a membrane

is a function of the size (or speed) of a particle in solution. By a careful selection of membranes a mixed solution may be separated into its constituent solutes. In general, a membrane acts like a filter-paper made infinitely fine—so that ultramicroscopic particles may be retained on the filter. Indeed, the process of separating substances in solution from one another has been termed " ultra-filtration " (p. 84).

Alterations in Permeability.

A living membrane, however, alters in its permeability. It may at one time allow a solute to pass through and at another prevent its passage : or at times allow a comparatively large particle to pass through while retaining smaller particles. It may also appear to " select " certain constituents of the surrounding fluid to pass in, seemingly quite irrespective of their size compared with their fellows.

Consider for the moment the passage of material across the membrane of the erythrocyte. On the one side we have the corpuscular contents, viz. haemoglobin, potassium, phosphorus and small quantities of calcium, glucose, etc. ; and, on the other side, the blood-plasma richer in water, proteins and salts than the corpuscle. Haemoglobin is freely soluble in plasma, but is held in greater concentration than would readily dissolve in the volume of fluid within the corpuscle (Chap. XXII.). The corpuscle also retains certain salts, organic and inorganic, in very different concentrations from those in which they occur in the plasma. Now glucose, chlorides and phosphates under certain conditions may pass easily in or out of the corpuscle, while sodium and potassium cannot permeate. Even when the plasma is diluted with water, provided the haemolytic concentration is not reached, the contents of the corpuscle are retained. If various samples of the plasma in which erythrocytes are suspended are diluted with different solutions isotonic with the plasma, *e.g.* (*a*) sodium chloride, (*b*) glucose, (*c*) urea, (*d*) ammonium chloride, it will be seen that the cell is not impermeable to all alike. The urea and the salts of ammonia with sufficient water to keep them in solution will pass into the cell, while the sodium salts will be kept out.

M. H. Fischer has demonstrated with characteristic clearness that the interface between a water-in-x phase and an x-in-water phase shows differential permeability, where x is a hydrophilic colloid, or even a hydrophilic substance like phenol, quinoline, or ether. Some coloured substances (such as neutral red, nile blue, methyl violet, methyl green) all leave the x-in-water phase, and are concentrated in the water-in-x phase so much that after a few

hours they may be found almost entirely in this phase. Iodine and eosin and similar substances pass in the same direction, but more slowly and less completely, while ferric chloride, cupric acetate, etc., take a very long time to leave the aqueous phase. On the other hand, the interface seems quite impermeable to the chlorides of cobalt, chromium and nickel. All the salts to which the living cell is impermeable are also excluded completely, or almost so, from passage across this laboratory-made interface.

Of course a cell is in close juxtaposition to several other cells, and therefore the composition, structure, and permeability of any one cell membrane may vary from place to place according to the nature of the interface. One interface may be such as to allow free passage of solutes to which other interfaces may be semi-permeable.

Alterations in permeability may be due to (1) alterations in the membrane or (2) alterations in the material presented to it from either side.

(1) The nature of the membrane is of great importance in studies of permeability. The cell membrane is unique, and one cannot guard too carefully against the adoption of generalisations drawn from experiments in which collodion, parchment or other artificial membranes have been used. Even the behaviour of dead animal membranes or of that of the erythrocyte is quite different from the true plasmahaut. Further, the membrane itself may undergo change in composition and permeability as the cell contents or the environment change in (*a*) composition, or in (*b*) physical state. The composition of the surface layer depends on the substances present in solution in the interior and on the nature of the interface. Any alteration in the chemical state of either of these phases will produce such an alteration at the surface as will alter permeability. The electrical double layer on the surface plays a considerable part in deciding the composition of the membrane. If a solute of opposite electrical sign to the membrane come within the electrical sphere of attraction it will be adsorbed and will either thicken the membrane or may occlude, wholly or partially, some of the interstices. In any case, adsorption will alter the permeability of the adsorbing surface. It may have a further effect. The adsorbed material may enter into combination, chemical or physical, with the membrane, producing a second alteration in permeability. It may even cause a third alteration, by ultimately passing through the membrane and going into solution on the other side.

If the adsorbed material be an amphoteric colloid, then the electrical charge on the membrane may be modified and so produce

apparently abnormal osmosis. Collodion membranes, for instance, are practically indifferent as regards electrical charge. Water passes through these membranes into solutions of non-electrolytes and of electrolytes more concentrated than $M/8$ at a rate in accordance with the van't Hoff theory of osmotic pressure, *i.e.* a linear function of the concentration of the particles (colloidal aggregates, molecules or hydrated ions) in solution. Treatment of the membrane with an amphoteric colloid like gelatine or haemoglobin causes an anomalous osmotic pressure. These colloids, as we have seen, form salts with either acids or bases. One may prepare, for instance, gelatine hydrochloride or sodium gelatinate. In the first instance, cationic gelatine has a + charge, while in the second case it acts as an anion and so has a − charge. The result of this is that when the membrane has a positive charge it will attract water as if the water had a negative charge, and *vice versâ*. That is, the rate at which water will pass through the membrane will depend on the intensity of the charge in the membrane, not on the sign of the charge.

(2) The material presented to the membrane may undergo changes :

(i.) Its particles may be increased in size,

(*a*) by adsorption of other material,

(*b*) by combining with similar particles,

(*c*) by hydration.

An increase in size, if sufficiently great, will prevent passage where previously passage was free.

(ii.) The converse may take place, *i.e.* the particles may be dissociated and so be able to pass through interstices previously too narrow for them.

(iii.) The electrical state of the material on either side of a membrane may undergo alterations. This is a general statement in which is included the effect of alterations of hydrogen ion concentration on permeability. The diffusion of water through an indifferent membrane depends on two forces, (*a*) pure osmosis, (*b*) electrical osmosis caused by the presence of electrolytes. *The intensity of the electrical forces depends on the nature of the electrolytes.* Neutral salts of mono- or di-valent cations influence the rate of diffusion as if they conferred a positive charge upon the water molecules. In other words, the molecules of the pure solvent are attracted by the charge on the anions and repelled by the charge on the cations of the electrolyte, the attractive and repulsive forces increasing with the valency and inversely with the radius of the ion. Alkalies act in the same way. If, however, one considers neutral and acid salts of tri- or tetra-valent cations, then one finds

the reverse to be the case. The water molecules act as if they were negatively charged and so are attracted by the cations and repelled by the anions of the electrolyte. Acids act in this way and have a high electrostatic effect on account of the small ionic radius of the hydrogen ion. It is important to note that certain salts of biological interest have a marked electrostatic value—very dilute solutions of oxalates, phosphates, and citrates and of the tetravalent ion $Fe(CN)_6$ attract water violently. On the other hand, the effect of the anion may be masked by the opposite electrostatic effect of the cation. As the valency of the cation increases, the attractive force of the anion decreases. Calcium chloride, for instance, has little more action than distilled water, because the calcium almost neutralises two positive charges on the two anionic charges (cf. Hydrophilic property of Ca, Chap. IX.).

The value of this electrical force has been determined by Loeb in a very neat manner. Inside a collodion bag he placed an $M/128$ solution of KCl and outside the bag an $M/64$ solution of sugar. These solutions are approximately isotonic, *i.e.* movement of water through the membrane by osmotic forces is thus eliminated. He found that water did diffuse from the sugar solution to the KCl solution. This transport of water must be due to the electrical pull of the KCl. He then raised the concentration of the sugar outside the bag till its osmotic pressure just balanced the attractive forces of the KCl. The sugar solution was now $M/8$. Therefore the electrical forces which are at work correspond to an osmotic pressure which is the difference between the osmotic pressures of an $M/8$ and an $M/64$ solution of sugar $= \frac{7M}{64} = \frac{7 \times 22\cdot4}{64} = 2\cdot4$ atmos. (approx.).

These electrical forces also account for **negative osmosis**—the passage of water from a more to a less concentrated solution. As far back as 1835, Dutrochet observed that water diffused out of a pig's bladder filled with a dilute solution of oxalic acid, into pure water. Early investigators tried to explain this on the assumption that there was a greater imbibition of water on the acid side of the membrane and a lesser on the side in contact with the pure water. In 1914, negative osmosis was observed taking place through a porcelain filter, and therefore the imbibition theory becomes untenable. Loeb has shown that negative osmosis occurs when neutral salts as well as acids and alkalies in certain well-defined concentrations are separated from water by a membrane capable of taking up either a positive or a negative charge. At these concentrations the repelling action of the ion with the same sign of charge as that of water becomes greater than the

attractive action of the ion with the opposite charge. The appended curve (Fig 34, from Loeb) shows the effect of concentration on the attractive force of Na_2HPO_4 on water. Various concentrations of this salt from $M/8192$ to $M/8$ were put into collodion bags fitted with a manometer. The ordinates are the values for the rise in the level of the solution in the glass tube (after the first twenty minutes) which occurred when the collodion bags filled with different concentrations of disodium phosphate were

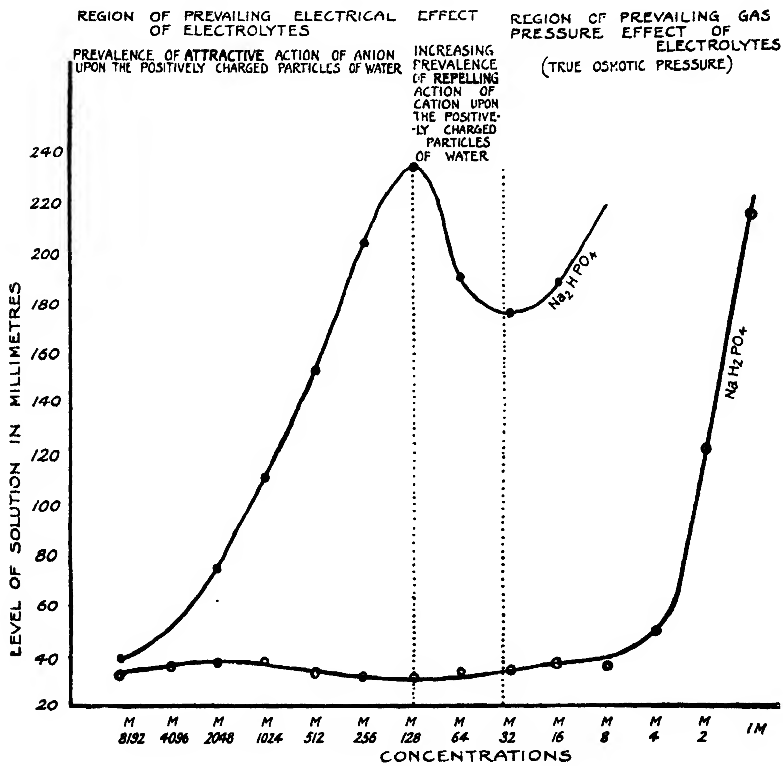


FIG. 34.

dipped into beakers of distilled water. The abscissae are the logarithms of the concentrations of the phosphate solutions. This curve shows clearly that at a very low concentration of the salt the rate of diffusion of water from pure solvent into the solution through the collodion membrane increases rapidly with increasing concentration, and that it reaches a maximum at a comparatively low concentration of the salt, viz.: $M/128$. This increase in rate has been shown to be due to the predominance of the attractive action of the anion upon the positively charged hydrols. With an increase in concentration beyond $M/128$ the rate of diffusion

falls abruptly to reach a minimum at a concentration of $M/16$. This fall is caused by the increasing prevalence of the repelling action of the cation on the positively charged particles of water. Further increase in concentration causes an increase of rate of diffusion. This final passage of water into the solution is due to true osmotic pressure. *At the concentrations where the rate of diffusion is decreased, i.e. where the curve falls* ($M/256 - M/16$ *in the case of* Na_2HPO_4) *water passes from the solution through the collodion membrane to the pure solvent.* That is, negative osmosis takes place.

Negative osmosis is a particular instance of electrical osmosis.

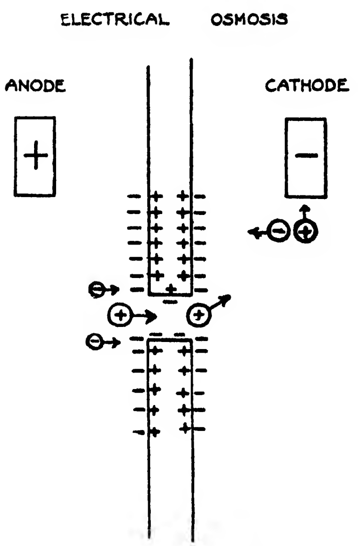


FIG. 35.—Diagrammatic section through a gelatin-collodion membrane showing a single large pore to demonstrate electrical osmosis.

In Part II., p. 534, will be found details of an experiment which shows that water can be drawn through certain colloidal membranes by direct electrical means. If the water is acidulated the attraction is towards the anode, but if alkali is added the water rises in the tube containing the cathode. To obtain this result, the membrane used must be of material capable of combining either with anions or with cations—*e.g.* of protein. Collodion does not form such compounds and so cannot form a membrane suitable for experiments on electrical osmosis until it has adsorbed an amphoteric colloid. In Fig. 35 is represented a gelatin - collodion membrane in acidulated water —*i.e.* in water with a slight excess of hydrogen ions. The membrane adsorbs some of these excess ions, interacts chemically with them to form gelatin hydrochloride, and so acquires a positive electrical charge. The passage of a current through the membrane and water depends on the carriage of the charge by ions—in this case H^+ and OH^-. The negative ions are attracted to the positively charged membrane till the charge on it is equalised. The positive ions attracted by the negative potential pressure at the cathode pass through the membrane, and raise the hydrostatic pressure on the cathodal side. It is obvious that the hydrogen ion concentration must increase at the cathode and decrease at the anode. (Pole finding paper is blotting paper soaked in phenolphthalein—an indicator which while colourless in neutral solutions becomes red in distinctly alkaline solutions.)

Water passes through the membrane in the reverse way when the solution on both sides of the membrane is alkaline. A dilute acid solution separated from a dilute alkaline solution of the same relative strength by an amphoteric membrane will produce a passage of water from the anodal to the cathodal side due to the greater speed of the positive ion.

Whatever causes may be assigned to the variations in permeability of plasma membranes, we can definitely exclude (*a*) mere variations in the size of the molecules presented to the membrane. Metallic cations, we have seen, are not allowed to pass through, while relatively large molecules like those of amino acids and glucose are able to make their way into the cell, and urea easily passes in or out, depending on which side of the membrane it is most concentrated. Hamburger and others thought that they had disproved Gürber's work on the lack of penetration of sodium and potassium. Recent work, however, has confirmed the view given above.

Bayliss separated a concentrated from a dilute solution of the sodium salt, congo red, by a membrane of parchment paper which is permeable to the sodium ion but not to the anion. He found that the dilute side became electro-positive on account of the preponderance of cations on that side.

Donnan (1911) propounded a theory of membrane equilibrium which is now classical. He studied a system such as that above where a membrane separated two liquids, in one of which was an ion (like the anion of congo red) which could not pass through the membrane. From thermodynamic considerations, he deduced formulae quantitatively connecting the ion concentrations on either side of the membrane (when equilibrium had been reached) and, by pure reasoning, predicted the presence of a potential difference across the membrane, the magnitude of which depends on the difference in the concentrations of the *diffusible* ions on both sides of the membrane. That is, if a solution of congo red (NaR) is separated from a solution of sodium chloride (NaCl) by a membrane impermeable to the anion of the dye (HR), the Na coming from the NaR would be prevented by electrostatic attraction from wandering far from its anion, *i.e.* it could not pass through a membrane except in minute quantities. The anion may be likened to a large dog attached by a short leash to a terrier (cation). The terrier could (and probably does) pass through between the rails of a fence, but is prevented by its large companion from penetrating far from the boundary. If we had a large number of such pairs, the chance of finding (at any particular time) a fair proportion of terriers in the field would be small. Two small dogs,

a terrier and a spaniel, on the other hand, similarly united might pass into the field together. One would then find that the concentration of terriers on either side of the fence would bear a definite relationship to the number of spaniels on that side.

Following Donnan's plan and indicating the membrane by a vertical line and molecular concentrations by square brackets, we have, to begin with :

$$(1)\qquad \begin{array}{r|l} Na^+ & Na^+ \\ R^- & Cl^- \\ (i)\ \text{inside} & \text{outside}\ (o) \end{array}$$

The NaCl diffuses readily into the cell, so that when equilibrium is established we have :

$$(2)\qquad \begin{array}{c|c} Na & Na \\ R & \\ Cl & Cl \end{array}$$

It can be calculated that at this point

$$\frac{[Na_i]}{[Na_0]} \times \frac{[Cl_i]}{[Cl_0]} = 1$$

Since the concentration of the cation Na inside the cell must be equal to the sum of the anions (R and Cl) present in order to maintain electrical equilibrium, whereas on the outside the concentration of Na is only equal to that of Cl, it follows that

$$[Na_i] > [Na_0] \text{ and } [Cl_i] < [Cl_0]$$

i.e. there is more cation (positive charge) inside the cell than outside. This difference of electrical potential is intensified by the smaller amount of mobile anion (negative charge) inside than outside. The potential difference thus created is balanced by the osmotic energy developed in the opposite sense, *i.e.* the energy which can be gained in this way is zero. Donnan has shown that the potential difference developed is

$$58 \log \frac{[Na_0]}{[Na_i]} = 58 \log \frac{[Cl_i]}{[Cl_0]} \text{ millivolts (at room temperature).}$$

Where the amount of NaR is large compared with the amount of NaCl, the P.D. would be 58 log [NaR]/[NaCl] millivolts.
where [NaR] = initial concentration inside the cell
and [NaCl] = initial concentration outside the cell.

As a matter of fact, as we shall see in Chap. XXIII., other balancing factors come into play as well (*e.g.* hydrostatic pressure).

Since many compounds in the cell are of the form NaR, and, as readily diffusible substances and membranes permeable to them abound both in the cell and forming a boundary to the cell, it

follows that potential differences due to the Donnan equilibrium are common occurrences in the body.

Differential permeability does not afford a sufficient explanation for all bioelectric phenomena. Even when the membrane is freely permeable to both ions of the salt and when the anion is the faster of the two, the dilute side of the parchment becomes electro-positive. This brings us again to the charge on the membrane. Parchment has a negative charge in water, and in dilute solutions of neutral salts—so has baked clay, wood, bone, charcoal, natural gelatin, etc., and all these cause a positive charge to develop on the dilute side. That is, the generation of a potential difference is just the reverse of electrical endosmose (Fig. 35). This may be confirmed by altering the charge on the membrane. Gelatin may be induced to take up a positive charge. Mines found that when a dilute solution N/80 was separated by a gelatin membrane from a more concentrated N/8 solution of sodium chloride, the dilute solution became electropositive. Gelatin is made positive by treatment with the ions of polyvalent metals. When an electropositive gelatin membrane was used, the dilute solution became negative.

A slight alteration in hydrogen ion concentration occurring on one side of a membrane will cause the development of E.M.F. If two solutions, one of *pH* 7 and the other of *pH* 8, are separated by a membrane more permeable to H^+ ions, an E.M.F. of about 30 millivolts may be obtained. The living cell has a *pH* of about 7·4 and its E.M.F. is not usually greater than 30 millivolts.

Polarisation. When a current is passed between two electrodes immersed in an aqueous solution, the potential difference between the electrodes tends to decrease and will in time fall off altogether on account of the deposition of ions of the opposite sign on the surface of the electrode. This polarisation of the electrode may be prevented by physical or chemical means (cf. various types of concentration cells). A similar ionic layer forms on membranes when a current is passed through them for some time (see also Chap. XII., Polarisation Current).

Selective permeability of membranes has often been noticed in electrical transference experiments. The classical experiments of Hittorff are now known to be, in some cases, vitiated by his use of ox-gut membranes to prevent convection currents. For instance, such membranes are much more permeable to SO_4 ions than to Cu ions. A large error is thus introduced into electrical diffusion experiments with $CuSO_4$ due to the adsorption of the copper ions on the substance of the membrane.

Till more is known of the physical state of the cell and its

environment, definite statements cannot be made concerning the causes of alterations in permeability of membranes. Phrases like " selective " adsorption should meanwhile be avoided, as they postulate intelligence in the cell to " select." Although the unknown must be explained in terms of the known, the day is surely past when it is necessary to assume a Maxwellian " demon " or a cellular intelligence. It is certainly not unscientific to admit the possibility that the unknown is similar to the known or may be explained by analogy to known physical processes.

Further Reading

McClendon and Medes. " Physical Chemistry in Biology and Medicine." Messrs. Saunders.

CHAPTER XII

THE CELL

"The life of the cell is a dynamic equilibrium in a polyphasic system."
SIR F. G. HOPKINS.

"Citizens of the state which the entire multicellular organism seems to be."

WE have seen that foodstuffs are broken down into units sufficiently small to pass through the intestinal wall. Logically, we ought next to study the system by which these absorbed substances are conveyed to the cell. It is important to realise that until they are inside the cell they are useless. *All energy changes take place in the living cell.* It will, however, be convenient first to examine a cell, note its imports and exports and study the various activities by virtue of which it is said to be alive.

In 1838, Schwann put forward the theory that animal tissues were an aggregation of large numbers of cells. Later work has justified this assumption. It is now generally held by biologists (1) that the earliest form of living matter was undifferentiated protoplasm, and that from this simple form of life there has been evolved, first the unicellular and then the polycellular organism; and (2) that each individual life follows this evolutionary course, originating as a single cell and gradually gaining in complexity with age. In view of these two beliefs, the evolutionary hypothesis (phylogeny) and the developmental history (ontogeny), it is logical to subject a unicellular organism to close examination in order that the various manifestations of life may be, at least, catalogued.

Amoeba is a unicellular animalcule which may be found in the stagnant water of almost any ditch. It is made of material differing so slightly in refractive index from the medium in which it lives that it can only be seen under the microscope after patient search. When seen it is found to be non-homogeneous. Apparently it consists of a greyish mass in which there are occasional granular aggregates, spaces containing water, spaces containing extraneous matter and a darker more compact mass, the nucleus. If the amoeba were cut in two the part which did not contain the nucleus would only live for a short time, while the other part would

function normally. The nucleus is, therefore, necessary for life. Ultramicroscopic examination shows that the grey mass is a hydrophilic colloid (emulsoid). Chemical analysis of dead amoebae confirms the ultramicroscopic examination. Water to the extent of about 75 per cent. is dispersed in the colloidal complex and acts as a solvent for certain crystalloids. The colloid is an aggregate containing protein, fat and carbohydrate. The crystalloids are to some extent adsorbed on the surfaces of the colloidal mass and to some extent are in free solution. Hofmeister estimated that a typical cell contains :

225×10^{15} molecules of water
53×10^{12} molecules of protein
166×10^{12} molecules of lipide
29×10^{14} crystalloidal molecules.

The elementary chemical composition conveys little information as to the properties of the complex. To say that protoplasm contains a certain percentage of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus in a colloidal state, and potassium, calcium, sodium, chlorine and phosphorus in solution is not of much use as a contribution to the study of life. It is just as preposterous to appraise the value of great pictures in terms of the chemical composition of the paints and pigments employed as to attach any great significance to the chemical elements of a dead cell. What is of great importance is the physical state of the matter, just as the value of a painting lies in the physical juxtaposition of pigments, an artistic blending of colour, light and shade, whereby the eye is pleased, so the life of a cell depends on the size, consistency, etc., of the colloid-crystalloid complex forming its protoplasm.

The water content of protoplasm is amazing in quantity and in physical state. We have seen (Chap. VIII.) that certain colloids have the property of imbibing large quantities of water and of holding that water under considerable compression (see also Experiment 39, Part II.). The physical properties of such water differ markedly from those of free water. For example, its vapour pressure will drop to a very low value and its removal from the colloid will be very difficult.

So, too, as has been pointed out in Chapter XI., is **the fat content** of the cell unique. Every cell has a fairly constant content of lipide, although when stained by the usual methods to demonstrate fat, no evidence is given of such a content. This masked fat is only made visible when the cell is diseased or disintegrated. The process of phanerosis or the unmasking of fat may

be likened to a change from a water-in-oil emulsion to an oil-in-water one (Chap. IX.).

The crystalloids, too, differ in their physical attributes from similar salts in solution. A salt solution isotonic with a 0·9 per cent. solution of sodium chloride exerts no osmotic effect either positive or negative on the mammalian cells, but has an electrical conductivity about five to thirty-five times as great as the cells. That is, the cell offers a greater resistance to the passage of an electrical current than its content of electrolytes would lead one to expect. If now, the cell is injured so that its contents undergo disintegration, its conductivity will approach that apparently justified by its composition. The high electrical resistance of living matter is due mainly to two factors—(1) the state in which the water is held, and (2) the adsorption of a large proportion of the electrolytes. (See also Chap. XXII., Blood.)

The water and a large proportion of the salts are dispersed through an apparently homogeneous colloidal mass. From ultra-microscopic examination one learns that the protoplasm varies in viscosity from cell to cell and more markedly from animal to animal. Some cells are almost liquid, as demonstrated by the vigour of the Brownian movement of the smaller granules in them, while other cells appear to be decidedly viscous with sluggish granular movements. The *annelid* egg exemplifies the former and the *sea urchin's* egg the latter state.

The viscidity of protoplasm may be influenced by alterations in the immediate environment. An excess of salts of monovalent elements, *e.g.* Na, K, guanidine leads to an increase in liquidity, while divalent cations produce the opposite effect. The presentation of a suitable mixture of mono- and divalent salts leads to the optimum viscidity of any particular cell. Loeb showed that *Fundulus* eggs, which were killed if placed in isotonic solutions of the chlorides of either sodium or calcium, would thrive in a definitely proportioned mixture of these two salts (cf. Salts and surface action, Chap. IX.).

The nucleus, as is mentioned above, is absolutely necessary for the continued integrity of the cell. It is, in *amoeba*, a spherical body, with a refractive index slightly higher than the cytoplasm and contains a nucleolus which is still more highly refractile. It also carries the chromosomes, the bearers or indicators of hereditary characteristics. The nucleus is surrounded by a membrane and is generally considered to contain a reticulum. Experiment has shown that the nucleoplasm reacts to salts much in the same way as the cell itself.

To sum up, the cell consists of three essential parts. (i) A mem-

brane, composed of lipide, protein and water; (ii) cytoplasm, a water-in-colloid dispersion; and (iii) the nucleus, a still more complex dispersion within its own membrane. All three elements are easily altered in physical state by alterations in the balance of electrolytes in the protoplasm or in the environment. The whole cell may be regarded as a polyphase crystalloid-colloid complex in unstable equilibrium. "When," says Sharpey-Schäfer, "the chemist succeeds in building up this complex it will, without doubt, be found to exhibit the phenomena which we are in the habit of associating with the term 'life'." What are the phenomena commonly associated with the term "life," especially as manifested by a unicellular animal?

(*a*) **Movement** is the commonest phenomenon indicative of life. Amoeba moves. It extrudes footlike processes, pseudopodia (Gr. pseudio, false (= similar to), podes, foot), at one part and retracts them at another and so moves along. Similar amoeboid movements are characteristic of the white cells or leucocytes (Gr. leukos, white) of the blood. Recently, Goodrich has carefully studied these movements of the leucocytes. He produces camera lucida drawings to show that the pseudopodia usually take up the form of expanded motile membranous folds when the living leucocytes are examined suspended freely in the normal fluid which is their habitat. One of his drawings is reproduced in Fig. 36. Movements of a precisely similar character may be produced in substances which are certainly not alive, such as Brailsford Robertson's model amoeba made of camphor, benzene and water (Part II.). These purely physico-chemical reactions are produced by alterations of the surface tension of the fluids under observation. Macallum has shown (pp. 173 and 185) that alterations in surface tension occur in living tissue during motion. Movement can, therefore, not be considered as a specifically vital phenomenon. Certain parts of the cell, *e.g.* the vacuoles, show a rhythm in their movements. In polycellular organisms, certain organs, *e.g.* the heart, pulsate. It is comparatively easy to produce rhythmical movement in material which is not living. A globule

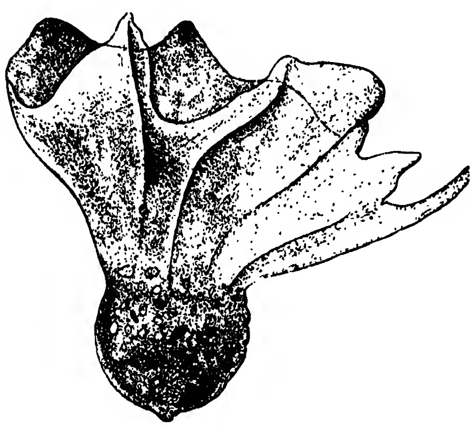


FIG. 36.—Leucocyte of invertebrate. (Redrawn after Goodrich.)

of mercury more than an inch in diameter may be made to pulsate with perfect regularity for hours. (See Ostwald's "Physical Heart," Part II.).

(*b*) **Irritability** is a general property of living matter. When amoeba is touched, it withdraws its pseudopodia (barotaxis) (Chap. XXXIII.). It moves towards and over suitable food and moves away from quinine or from a hypertonic solution of crystalloids (negative chemiotaxis). Hydrogen ions if not too concentrated exert positive chemiotaxis, while hydroxyl ions have a repellent effect. This may explain galvanotaxis. Strong light repels, while a moderate illumination attracts many lower organisms. Further, the more refrangible rays of light exert a negative phototaxis, while the less refrangible rays are positively attractive. If the swarm spores of certain algae are placed in a tank with a cover, half of which is blue glass and half is red, and exposed to light, they will stream away from the blue and towards the red end of the box. Ultra-violet rays have a marked effect on living organisms, for example, the tubercle bacillus is killed by ultra-violet light and lupus is cured by projection of the Finsen arc on the growth. Change of temperature may exert either a positive or a negative effect, the animalcule avoiding the abnormal. That is, too high or too low a temperature exerts negative thermotaxis. Non-living matter shows irritability. We have seen how sensitive colloids are to slight alterations in their environment. They exhibit chemiotaxis and galvanotaxis very markedly. Even inorganic matter may respond to stimulation. Lillie has demonstrated this in the case of iron. A piano wire which has been dipped in concentrated nitric acid and then suspended in dilute nitric acid will show changes if "stimulated" mechanically, chemically, or electrically. The irritability of living matter is, according to Verworn, of a specific type and is thus indicative of life.

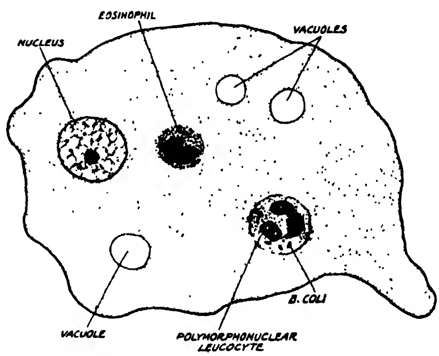


FIG. 37.—Large mono-nuclear cell (macrophage) from the peritoneal fluid of a rabbit suffering from peritonitis induced by inoculation with B. coli, showing phagocytosis of (1) a polymorphonuclear leucocyte, which has itself ingested some bacilli, and (2) an eosinophil leucocyte.

(*c*) **Ingestion and excretion** are phenomena exhibited by all living cells. Nutrient material is taken from the environment, prepared, used, and the non-utilisable rest is forced out. Amoeba engulfs food and forms a vacuole in which will be found food and

water. Into this vacuole are secreted digestive enzymes which reduce the ingested material if possible from the colloidal to the crystalloidal state. It then passes into the protoplasm and the undigested residue is forcibly excreted by contraction of the vacuole. These processes all have their physico-chemical counterparts. A drop of chloroform will reject a piece of capillary glass tubing forced into it. If the glass be coated with shellac it will be drawn into the chloroform, the shellac dissolved from it and then the clean glass be expelled from the interior of the chloroform to the surface.

(*d*) **Growth** is not a property characteristic of living matter. Leduc has taught us that by osmosis life-like forms may be produced which grow.

(*e*) **Electric Phenomena.** The electrical power generated by living matter has always been a subject of interest and of amazement. Quite apart from such animals as possess electrical organs, *e.g.* the electric eel which can generate an E.M.F. of several hundred volts, every living animal, in fact every living cell, produces electromotive forces. The ordinary potential differences observed in living matter may seldom reach 0·1 volt, but everyone knows that if *n* small units are connected in series, the resultant voltage is *n* times the voltage of the single unit. Dissection of an electric organ shows that it is built up serially of large numbers of units. The cause of the potential differences in cells must be sought for in the " selective " permeability of the cell membranes (p. 145), or in alterations of the content of the protoplasm in electrolytes.

Electrical effects are produced in living cells by suitable stimulation. If a cell is injured the injured part becomes electropositive to *the rest.* This phenomenon, apart from any other conditions, would be quite sufficient to justify the postulation of a cell membrane. Consider the cell as a mass of protoplasm in an envelope of matter which is permeable to the negative ion but not to the positive ion of a dissociated electrolyte. This will cause a difference of potential on the two sides of the membrane. Inside will be a preponderance of negative ions while outside will be an equal preponderance of positive ions (Fig. 38). **Current of Injury.**—If now we could connect the inside of the membrane with the outside, there would be a flow of current till the difference of potential had been equalised. Current would flow to the pierced, injured (or inside) part from the outside. That is, the injured part would be similar to the zinc pole of a zinc-copper galvanic couple. There the flow of current is from the zinc to the copper *inside* the battery. The zinc is therefore said to be electro-

positive. The current, however, flows from the copper to the zinc, *outside* the battery. The zinc is, therefore, said to form the negative pole. **Current of action.**—When alterations of tension or stress take place in a cell they are accompanied by alterations in electric potential. The part under stress becomes as if injured, *i.e.* electropositive or zincy to the normal or unstressed part. This may be due to an increase in the permeability of the membrane at the stressed part, so that the positive ion gains access to the cell. The seat of stress does not, however, remain at its point of

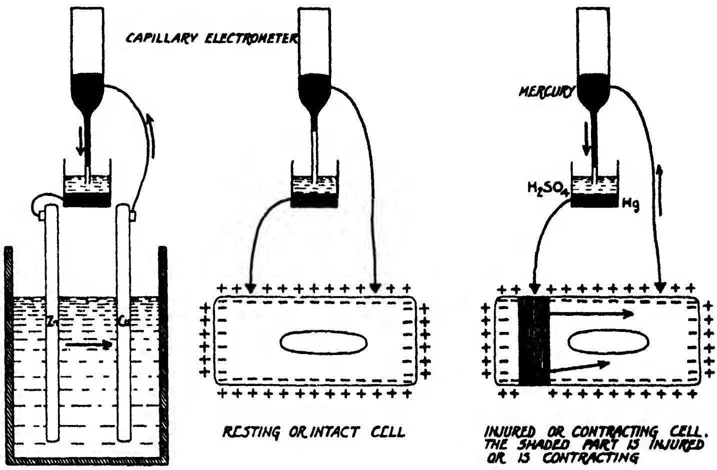


FIG. 38.—To show the origin and method of measuring the current of action or of injury in a living cell. In the resting cell (central figure) all points on the surface of the cell are at the same potential. Note position of Hg in the capillary tube. Action or injury produces contact with the interior of the cell at the part active or injured, so that a current flows with the effect shown. Compare with the zinc-copper galvanic cell.

origin, but passes as a wave of increased stress over the whole cell followed immediately by an electropositive wave (Fig. 43).

MacDougal and Moravek (1927) have constructed a cell that answers several of Sir Edward Sharpey-Schäfer's requirements (p. 150). Their cell membrane consisted of a Soxhlet thimble (cellulose + calcium salts) impregnated with a solution of *cholesterol* in *lecithin*, which mixture is incorporated in a hydrophilic gel (gelatin-agar). Various mixtures, solutions, etc., may be put inside the cell and the cell immersed in various solutions. For example, when the cell contained 20 per cent. sugar solution, an increasing *endosmotic* series in Na > K > Ca is given, implying decreasing permeability with this series of cations. Further addition of any of these cations further decreases permeability. The cell wall shows excellent buffering power (Chap. XXII.)—*i.e.*, even when the hydrogen ion concentration of the external solution varied as much as from pH 3·05 to pH 8·2, the interior remained

practically neutral. This power is conferred on the "constructed cell" by the *lecithin* incorporated in its membrane.

Polarisation Current.

In all chemical processes, alterations in potential difference take place. The living complex, known as a cell, is a system in which chemical transformations proceed continually and, therefore, electromotive force is being generated continuously. These currents may be demonstrated if special arrangements are made to prevent polarisation of the electrodes (Part II.). The electrodes which are used to lead the current from the cell (or group of cells) to the galvanometer are subject to polarisation, as explained in Chap. XI. The products of electrolytic decomposition of the cell substance are transported to the electrodes and accumulated there. The deposition of these products at the two poles, in course of time, alters the nature of the electrodes. The cathode, for instance, because of the accumulation of positive ions on it, becomes more and more anodal. This produces an electric tension that causes a current, the so-called polarisation-current, to flow in the opposite direction to the original one. As this current grows in strength it reduces the value of the tissue-current, and after a short time completely obliterates it.

FURTHER READING

McCLENDON & MEDES. "Physical Chemistry in Biology and Medicine." Messrs. Saunders.

CHAPTER XIII

RADIO-ACTIVITY

THE ATOM IN DISSOLUTION

"From harmony, from heavenly harmony
This universal frame began;
When nature underneath a heap
Of jarring atoms lay."

DRYDEN.

THE various manifestations of energy already dealt with have all been associated with matter in the form of small aggregates (colloids), atoms, or ions (charged hydrated radicles). Chemists once defined the atom as the smallest non-divisible portion of matter. Needless to say, many scientists were content to be decryed as old-fashioned and refused to accept this opinion of the atom. My old teacher, Prof. John Ferguson, would allow no one to refer to atoms. He preferred the more cumbrous but exact term "Combining Proportions." Modern work has confirmed these opinions of the atom. Physicists are now interested in the structure of the atom. No longer is it considered as non-divisible. No longer does it remain as fundamental. Of what then does the atom consist? Many and varied are the present-day theories of its structure, but in general most schemes are similar. It is supposed to consist of a number of smaller units—electrons, all moving rapidly, eccentrically and *regularly* round a central positive charge or nucleus. An electron is nothing more than a unit charge of negative electricity. The number of electrons in each ring is definite and may undergo alteration in definite quanta only.

(1) Not more than a certain number of electrons can continue in stable motion in one ring. If more are added the system breaks up into two or more rings.

(2) If the orbital velocity of the electronic rings exceeds or falls below a certain critical value, the electrons are rearranged to ensure stability for that speed.

A model may make this clearer. The outer particles may be represented by a number of exactly similar sewing-needles, magnetised simultaneously in a solenoid. They are floated vertically in a small trough (Fig. 39), by having, say, their N poles

inserted each to the same depth in exactly similarly pieces of cork. The place of the positive core is taken by a bar magnet set vertically, *N* pole upwards, below the trough. It will be noticed that the needles arrange themselves in two rings. If ten needles are floated, seven will be in the outer ring and three in the inner.

It has been found that the greatest number of magnets which we can have in an empty ring is five. If a sixth is added, two rings are formed with five needles on the outside and one in the middle. The number which must be placed in the middle rapidly increases

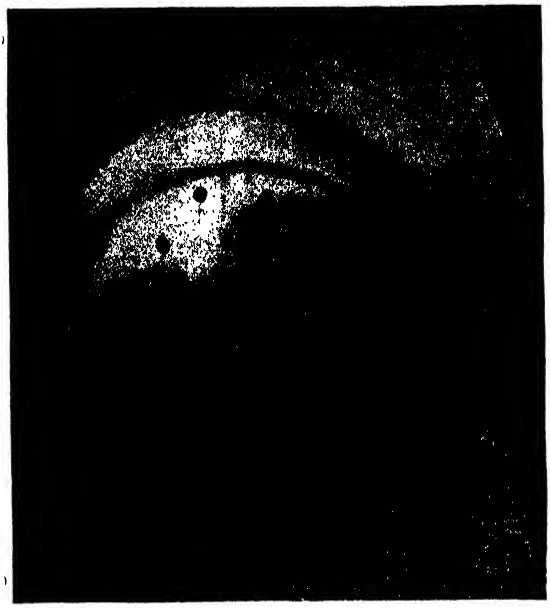


FIG. 39.—A "Model" Atom. (From Crowther's *Molecular Physics*.)

with the number in the outer ring. The removal of one needle from the outer ring may cause a complete rearrangement of the needles, *i.e.* a new series of concentric circles may be formed differing from the first series in the number of the component needles in each ring.

As we have mentioned, the electrons forming an atom are supposed to be in constant rapid and eccentric motion. If for some reason the motion of an electron becomes centrifugal, then, if sufficient speed be developed, it will tend to fly off. The atom will be ruptured and a new atom will be formed.

In 1878, Crookes found that the passage of an intermittent high-tension current of electricity through a tube from which air had been so thoroughly withdrawn that only about 10^{-7} of atmo-

spheric pressure was present, produced the so-called *Kathode Rays.* These rays originate at the kathode at right angles to its surface and proceed in straight lines like light independent of the position of the anode. Whatever comes in the path of the rays is caused to fluoresce, *e.g.* the walls of the tube. They heat the object struck. By using a concave kathode, they may be focussed on a piece of platinum, which soon becomes red hot, and may even be fused. Mechanical pressure is exerted by the rays. If directed on to light vanes attached to an axle they may be made to turn little mills, or in the " railway tube " they drive a wheel along glass rails. The stream of rays is deflected by a magnet as if it were a stream of negatively charged particles. In 1893, Lenard, following up Hertz's discovery that metal was transparent to the kathode rays, made a small window of aluminium foil in the end of the vacuum tube and so brought the kathode rays through the foil into the open air.

In 1895, Roentgen, repeating Lenard's work, accidentally discovered the X-rays. He had covered the vacuum tube with a black paper case to shield the eyes from the kathode fluorescence, so that the effect of the rays outside the tube might be more easily observed. He thus noticed that a barium-platinocyanide screen which happened to be near became fluorescent whenever the tube was working though no visible rays could reach it. On placing his hand between the screen and the tube, he saw, for the first time, the now familiar sciagraph of the bones of the hand. The X- or Roentgen rays originate from the place where a kathode ray strikes, from the walls of the tube, in the first instance, or in a focus tube from the piece of platinum (anti-kathode) upon which the kathode rays are focussed. They issue equally in all directions and travel in straight lines. For any tube, the power of penetration of the X-rays is inversely proportional to the density of the substance penetrated. The higher the degree of exhaustion of the tube the greater the penetrating power of the rays produced. In a " hard " tube the vacuum is so good that a very great difference in potential between the electrodes is necessary to force the discharge through. The kathode rays therefore attain a high velocity and the X-rays they produce on impact with the anti-kathode have a high penetrating power. On the other hand, if the tube is not well " exhausted," the X-rays evolved are easily absorbed. Such a tube is termed " soft." Unlike the kathode rays, they are not affected by the most powerful magnetic field. Like the kathode rays, they excite fluorescence, act on sensitised photographic plates and ionise gases, *i.e.* they make air, or other gas through which they pass and which under ordinary circum-

stances are practically insulators, capable of conducting limited quantities of either positive or negative electricity.

Poincaré suggested that the production of X-rays might be an effect common to all fluorescence. In 1896, Becquerel, acting on this idea, examined some fluorescent salts of uranium. He found that the double sulphate of uranium and potassium exposed to sunlight could affect a sensitised plate even when the plate was protected by a layer of copper or aluminium foil. This metallic layer excluded the possibility of action by ultra-violet light or by chemical vapours emitted by the salt. Further investigation showed that the phenomenon was exhibited by uran*ous* salts which are not fluorescent, as well as by the fluorescing uran*ic* salts. Both are active in proportion to the amount of uranium they contain. That is, the continuous emission of these rays is a specific property of uranium now generally termed **Radio-activity.**

The characteristics of the radiation from uranium are very similar to those of the X-rays. They are found to consist of three very distinct types of rays, differentiated in the first instance by their power of penetrating matter. They have been termed by Rutherford, α, β and γ rays. The α rays are particles of the gas helium expelled radially from the uranium with the colossal speed of 20,000 miles a second. They have so feeble a penetrating power that they are completely stopped by a single sheet of note-paper or by about 7 cm. of air. The α rays carry a positive charge, but are only slightly deviable by an intense magnetic field. The β rays resemble the X-rays in penetrating power, and pass with ease through thin metal, glass, etc., but are nearly all stopped by a single coin. Becquerel proved that the β rays are identical with the kathode rays, *i.e.* electrons. Their superior penetrating power is due to their enormously greater velocity. The γ rays are not deflected by magnetic fields. They resemble in all respects the X-rays, but are far more penetrating than rays even from the hardest vacuum tube. They will readily pass through a pile of twelve coins. Their nature is probably the same as that of X-rays, *i.e.* thin pulses in the ether.

The α and β rays do not penetrate gases by pushing aside those molecules of the gas that lie in their path. They actually pass through the molecules (which, of course, are mostly " hole ") and knock off, in their progress, some of the outlying electrons. In passing through 7 cm. of air, the α particle chips off about 130,000 electrons and so " ionises " the air.

This power of ionising a gas is used as a means for measuring the intensity of radiation. The simplest apparatus for this purpose is

a gold-leaf electroscope. Fig. 40 represents the type of electroscope used by Soddy. It consists of a tin can with a movable bottom *E* for the insertion of the substance to be tested. A paraffined rubber cork, *H*, is pierced in the centre by the metal wire, *G*, which carries at its end a rod of fused quartz, *A*. A thin brass strip, *B*, to which a single gold leaf, *C*, is attached, is fastened to the lower end of the quartz rod. *F* is a vulcanite handle by means of which the charging rod, *D*, can be brought into contact with *B*. The rate of collapse of the gold leaf may be observed by means of a reading microscope through a window in the can (dotted line).

In 1903, the Curies, who were examining the minerals containing uranium, discovered a new element, radium, in pitchblende. This very radioactive material was obtained pure in 1911. From a ton of pitchblende may be extracted about 200 mgrms. of radium chloride, which was responsible for over 80 per cent. of the radio-activity of the raw material.

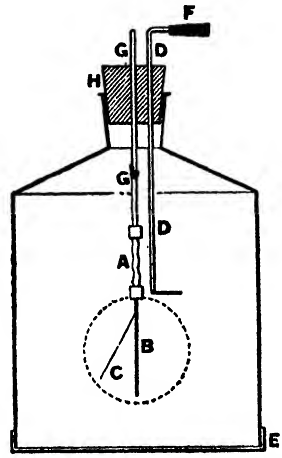


FIG. 40.—Section through gold-leaf electroscope as used to determine the ionising power of radio-active matter. See text. (*From* Soddy's *Radioactivity*. Electrician Press.)

Subsequent investigations by the above workers, and principally by Rutherford, Soddy, and their collaborators, have shown that there are three series of radio-active elements. The appended chart from Soddy shows the relationship between the members of the series and between two of the series themselves. This chart demonstrates to us the remarkable fact that the atom of the heavy elements at the head of each series is continuously and regularly undergoing disintegration. Matter and energy are being lost at a rate which, so far, cannot be modified in any way.

Lately Campbell and Wood have discovered that certain of the elements of low atomic weight are also radio-active. One of these, potassium, is found universally and in abundant quantities in animal and vegetable cells. Potassium is a necessary permanent constituent of every living cell. Of the 12–15 elements essential to life, it is the only one possessing distinct if minute radio-activity. The activity of potassium may readily be demonstrated by means of the gold leaf electroscope. It is shown that β rays are emitted. Potassium is 1,000 times weaker than uranium and 1,000,000,000 times weaker than radium in the emission of β rays.

TABLE XXIII

TABLE OF RADIO-ACTIVE DISINTEGRATION.*

I. MAIN SERIES.

A. URANIUM, RADIUM AND ACTINIUM SERIES.

Element.	Atomic Weight.	Radiation.	Period of Average Life.	Chemical Character (Isotopic with).
Uranium-I.	238	α	8,000,000,000 years	Uranium.
Uranium-X¹	234	β	35·5 days	Thorium.
Uranium-X²	234	β	1·65 minutes	Eka-tantalum.
Uranium-II.	234	α	3,000,000 years (?)	Uranium.
Ionium	230	α	100,000 years	Thorium.
Radium	226	α	2,440 years	Radium.
Ra. Emanation	222	α	5·55 days	Emanation.
Radium-A	218	α	4·3 minutes	Polonium.
Radium-B	214	β	38·5 minutes	Lead.
Radium-C	214	β (99·97%)	28·1 minutes	Bismuth.
Radium-C′	214	α	1/1,000,000th sec. (?)	Polonium.
Radium-D	210	β	24 years	Lead.
Radium-E	210	β	7·2 days	Bismuth.
Radium-F (Polonium)	210	α	196 days	Polonium.
End Product	206	—	Infinitely long	Lead.
		B. THORIUM SERIES.		
Thorium	232	α	25,000,000,000 years	Thorium.
Mesothorium-I.	228	β	9·67 years	Radium.
Mesothorium-II.	228	β	8·9 hours	Actinium.
Radiothorium	228	α	2·75 years	Thorium.
Thorium-X	224	α	5·25 days	Radium.
Th. Emanation	220	α	78 seconds	Emanation.
Thorium-A	216	α	0·2 second	Polonium.
Thorium-B	212	β	15·4 hours	Lead.
Thorium-C	212	β (65%)	87 minutes	Bismuth.
Thorium-C′	212	—	1/100,000,000,000th sec. (?)	Polonium.
End Product	208	—	Infinitely long	Lead.

II. BRANCH SERIES. (*A.*)

(At either Uranium-I. or Uranium-II. the series branches, and 8 per cent. of the total number of atoms disintegrating follow the branch Actinium Series.)

Element.	Atomic Weight.	Radiation.	Period of Average Life.	Chemical Character (Isotopic with).
Uranium-Y	—	β	2·2 days	Thorium.
Eka-tantalum	—	α	1,000 to 10,000 years (?)	Eka-tantalum.
Actinium	—	β (?)	(?)	Actinium.
Radioactinium	—	α	28·1 days	Thorium.
Actinium-X	—	α	16·4 days	Radium.
Ac. Emanation	—	α	5·6 seconds	Emanation.
Actinium-A	—	α	0·003 second	Polonium.
Actinium-B	—	β	52·1 minutes	Lead.
Actinium-C	—	α	3·1 minutes	Bismuth.
Actinium-D	—	β	6·83 minutes	Thallium.
End Product	Either 206 or 210	—	Infinitely long	Lead.

(At Radium-C, 0·03 per cent. of the atoms follow the branch series.)

Element.	Atomic Weight.	Radiation.	Period of Average Life.	Chemical Character (Isotopic with).
Radium-C	214	α	28·1 minutes	Bismuth.
Radium-C_2	210	β	1·9 minute	Thallium.
End Product	210	—	Infinitely long	Lead.

(*B.*)

(At Thorium-C/35 per cent. of the atoms follow the branch series.)

Element.	Atomic Weight.	Radiation.	Period of Average Life.	Chemical Character (Isotopic with).
Thorium-C	212	α	87 minutes	Lead.
Thorium-D	208	β	4·5 minutes	Thallium.
End Product	208	—	Infinitely long	Lead.

* (From Soddy.)

As is well known, potassium is an absolutely necessary constituent of the fluid used for the perfusion of an organ. If a potassium-free Ringer's fluid is passed through a frog's heart, the heart will come to a standstill in about half an hour. The frog's peripheral vessels may be perfused with Ringer's fluid for hours without any sign of oedema. As soon as a potassium-free fluid is used, marked oedema begins, causing the frog to swell and increase in weight. Further, the frog's kidneys when perfused with Tyrodé's fluid or similar fluid containing glucose allows no glucose to pass out into the urine. If the potassium is omitted in making up the fluid, glucose at once escapes into the urine. Ringer demonstrated, long before its radio-active nature was discovered, that rubidium may be substituted for potassium in equimolecular amounts. He explained this by its similar chemical nature. Similarly, caesium, another of the lighter radio-active elements, may take the place of potassium in the perfusion fluid. No non-radio-active element has been found which is capable of acting as a substitute for potassium. Further, Zwaardemaker was able to perform normal perfusions provided a substance emitting β rays was within effective distance of the frog.

The last-named worker and his collaborators then set out to determine the amounts of the heavy radio-active elements necessary to replace potassium. These radio-elements, as we have seen, emit α rays in marked excess of the β rays necessary for physiological purposes. They found that, as was to be expected, the α radiation completely masked the β radiation. If means were taken to exclude the α rays, these $\alpha + \beta$ radiating salts acted as excellent substitutes for potassium. Radio-active substances may thus be classified for biological purposes into two groups (Table XXIV.).

TABLE XXIV

I.	II.
β Radiating (negative).	α Radiating (positive).
Potassium.	Uranium.
Rubidium.	Thorium.
Caesium.	Radium.
	Ionium.
	Lanthanum.
	Cerium.
	Niton (Emanation).

A heart beating with a fluid containing the appropriate quantity of any of Group I. may be switched on to any other group I. element in æqui-radio-active amounts. Similarly, the Group II. elements are interchangeable. But direct transference from a I.

fluid to a II. or *vice versâ* at once produces complete stoppage. The two groups are antagonistic. If, however, the heart is washed completely free from one group with radio-active-free fluid it may without harm be perfused with a fluid containing one of the elements of the antagonistic group.

Fluorescein and eosin adsorb the α and β rays unequally. If one of these dyes be added to the perfusion fluid the amount of radio-active material present may be reduced appreciably and still produce normal action. In summer, smaller quantities of radio-active salts are needed than in winter. This is related to the lowered calcium content in the frog's blood in winter.

To summarise, potassium is a necessary constituent of all living matter because of its property of emitting electrons (β rays). It may be replaced by other radio-active substances in æqui-radio-active proportions provided these substances are not otherwise toxic. How potassium acts in the living cell can as yet be only a matter of surmise. Presumably the freed electron passing with great velocity through crowds of ions, molecules and colloidal aggregates will have some effect on them. It is known to have at least two effects :

(1) Because of its velocity, the β ray accelerates the rate of migration of gaseous ions in a similar way to ultra-violet light of extremely short wave-length (below 2,000 Ångström units), *i.e.* the gas becomes an electrical conductor.

(2) On account of its unit negative charge, it has a disturbing effect on all systems in electrical equilibrium through which it passes.

Rutherford has recently shown that α particles (positively charged nuclei of helium, atomic weight = 4) may cause trivalent nitrogen (14) to disintegrate with the formation of monovalent hydrogen (1). He considers that the hydrogen ion is a unit positive charge. Other atoms are composed, as we have seen, of a positively charged nucleus about which are grouped sufficient electrons to render the whole system neutral.

The modern tendency is thus to postulate the sameness of all elementary matter. What we have been accustomed to look upon as elements may merely be stages in the disintegration of more complex substances into their positive and negative units. When the disintegration takes place explosively and continuously the substance is considered as radioactive.

In the preceding portion of this chapter, kathode rays, X-rays and the α, β, and γ rays of radio-active matter have been mentioned as types of radiation. These various radiations differ from one another in their effects on living matter in degree rather than in

kind. In general, the lower the velocity of the ray, the greater its physiological action, provided always that its velocity is sufficiently great to produce any physiological effect. (A high velocity bullet cuts a clean hole in a piece of glass, while a spent bullet shatters the glass.) The effect seems to depend more on the velocity of the ray than on its nature, *e.g.* α rays have mass while the others have not, yet similar actions may be produced under proper conditions. The physiological effect of any ray is proportional to its power to ionise air. β rays have 60 times the ionising power of γ rays, and experiment has shown that γ rays require to operate for 60 mins. on living matter to have the same effect as one minute's exposure to β rays. That is, by varying the length of exposure, similar results may be obtained from radiations having different ionising powers. If rays are classed according to their power to ionise air, then those having the greatest ionising effect have an inhibitory rather than a beneficial effect on living organisms, while, conversely, the weaker rays promote the function of the organism. The power of ionisation being equal, then generally a long exposure produces inhibitory effects and a brief exposure beneficial. For example, exposure of the fertilised eggs of *arbacia* to rays of radium, if short, causes stimulation of the cell function. If the radium is applied during the approach of the germ nuclei, then cell division is accelerated. If the exposure is long, cell division is retarded. The effect of radium is more marked during the metaphase than during either the prophase or the telophase. Eggs are not so easily influenced by radium emanations after the dividing stage is passed. In order to produce any effect on the rate of growth of *ascaris* eggs about ten times the intensity of radium has to be applied as was effective during the dividing stage, or the length of exposure has to be increased tenfold. The β and γ rays seem to act on different parts of the egg. The nucleus, especially if it is undergoing mitosis, is influenced by the γ rays, while the β radiation has most effect on cytoplasm. The fertilisation membrane of *nereis* is thickened as a result of exposure to radium, the length of exposure being, in this case at least, more efficacious than the intensity.

The rays emitted by radio-active elements, especially radium, have been employed extensively in the treatment of morbid cell growths. The cells are not killed outright, but division of the nuclei is inhibited, eventually leading to death of the cell. The rays are capable of causing definite regressive changes even in deep seated tumours such as mediastinal lympho-sarcoma, carcinoma of the lungs, and abdominal metastases of carcinoma of the testis.

Several investigators have reported results to show that the

growth-promoting substance in yeast may be partially inactivated by exposure to radium emanation. It is probable that the therapeutic effect of radium treatment may be due to this destruction of the growth-promoting substance. It has been known for long that radium rays have a destructive effect on colloidal solutions, due probably to a disturbance of their electrical state. Globulin and vitellin are coagulated and lecithin suspensions are decomposed on exposure to radium emanation. That the action is electrical is borne out by the antagonism between α radiation and β radiation. Either of them prevent bacterial growth in agar cultures, but the simultaneous application of both kinds of rays is ineffective (cf. antagonism of colloids, etc., p. 96). Of course, normal as well as pathological tissue may be damaged by exposure to radium. The action is similar to exposure to cold. Radium causes an immediate decrease in the total number of white cells in the blood (Chap. XVI.). This result is probably due to inhibition of the *formation* of the leucocytes rather than to the destruction of already formed cells. The greatest decrease occurs from ½ to 6 hours after application of the radium. Within 24 hours a normal concentration of white cells may be observed.

By the operation of Le Chatelier's principle (*q.v.*), matter exposed to radio-active elements should develop some protective mechanism against the action of the rays. Becquerel noticed that β rays changed yellow phosphorus into its red form, which is not acted on by the rays. We have already mentioned that the fertilisation membrane of *nereis* is thickened where exposed to radium. Some observers find that the presence of chlorophyll is protective. Others deny this.

Ultra-Violet Rays.

The physician is interested in radiant energy of this type because of its lethal influence on pathological growths and on bacteria. Ultra-violet rays, or lights like the Simpson Light, which emit a large proportion of ultra-violet rays, have been employed as germicides in surface wounds. The penetrating power of these rays is slight, and, therefore, they can have little effect on deep-lying structures.

The tissues may, however, be made sensitive to rays of somewhat longer wave-length by administering coloured substances which act as sensitisers. For example, haematoporphyrin, an iron-free disintegration product of haemoglobin, so sensitises the tissues that ordinary daylight produces similar effects to ultra-violet light. These effects are mainly psychological and photochemical. The latter action has been carefully investigated, the

former is extensively exploited. Chief among the photochemical actions is that of forming a protective pigment in accordance with the principle of Le Chatelier. Some of the other chemical results of exposure to ultra-violet rays are of interest, but can only be mentioned here.

(1) Ergosterol has two well-defined absorption bands between 2,500 and 3,000 ÅU. After irradiation these bands vanish and new bands appear further in the ultra-violet region (about 2,100 and 2,400 ÅU). Irradiated ergosterol is extraordinarily effective in curing rickets, and is thus of use where effective sunlight cannot be directly applied to the body.

(2) Hydrophilic colloids on exposure to radiation "take on" some extra electrons, and so become internally more mobile. If they are incorporated in a membrane that membrane becomes more permeable.

(3) Certain syntheses are accelerated by the irradiation of the reacting substances, *e.g.* exposure to rays of 2,000–2,500 ÅU of a solution of ammonium carbonate for two or more hours causes the formation of urea. This synthesis is probably brought about by alternate reduction and oxidation of carbonic acid.

Further Reading

Russell. "Ultra-Violet Radiation." Messrs. Livingstone.
Millikan. "The Electron." University of Chicago Press.
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SECTION III: CELL COMMUNITIES

CHAPTER XIV

THE ARMY WHEREWITH THE BODY WAGES WAR WITH NATURE—THE MUSCLE CELLS

"Tho' born to fight,
Yet, mix'd and soften'd, in his work unite;"
POPE.

"Lactic acid is the keystone of the arch which now joins the physiology of muscle to the exact sciences."
A. V. HILL.

IN the animal body there are various kinds of cell communities. There seems to be no doubt that originally each cell was self-supporting, and a small cell-community, like a small village in a remote corner of civilisation, was able to perform all necessary activities without the help of other communities. In a big complex concern like the mammalian body, however, each cell community has specialised in some form of activity, and it has therefore to depend on other communities for certain necessities. No cell in such a community is absolutely self-supporting. For the same reason we cannot validly consider any cell as typical of all others. Each has its own particular duty to perform and is adapted to perform that particular duty most economically. It could and might, if circumstances compelling it arose, do other things usually left to other cells, but would perform these unaccustomed duties clumsily and uneconomically.

The dominant cell communities in the somatic body are those forming the muscles. Their activity, to a great extent, regulates all other changes taking place in the body. They demand for their use the lion's share of the energy intake of the body. The bulk of the repair material in the food is earmarked for their use. They keep a firm hand on the transport system and soon cause a "speeding up" if supplies fall short of their needs, or if the bye-products of their activity are not removed with sufficient rapidity. The system of inter-communication between cell-communities (the nervous system) exists in large measure to suit the muscles. In short, the muscles are the master-tissues of the soma. They

are not a manufacturing community but are power users. In another sense, the muscles are the servants of the body. By means of them, the body fights a war not merely of defence but of aggression against its environment. As civilisation has advanced, man has found it convenient by means of tools and machines to add power and speed to his muscle. By so doing, he has been able to harness and utilise power from sources that could not have been tapped otherwise. Tools and machines are thus extended and detachable limbs.

The Muscles are Energy Transformers. In the first instance, they act as accumulators *accepting* energy principally in two forms (*e.g.* potential energy of glucose and osmotic energy of glucose, phosphates, etc.) and then *storing* that energy in potential form (in a glycogen-nitrogen-phosphorus compound having a very low osmotic pressure) till it is required, when it is liberated (in what form we do not know) and converted into kinetic energy. That is, muscle is a compound transformer. (*a*) It stores energy and, just like any other accumulator, the *amount* of energy stored depends on the *size* and number of the units, and the *potential* of the energy released only on the *number* of units composing the muscle. When a muscle has its full charge, it can take no more. The amount of glycogen stored in muscle is definitely limited by the bulk of the muscle for each particular type of muscle. (*b*) On activation, it transforms portions of the stored energy into some form which acts on the liquid colloidal mass within the fibres, causing the mass to become less liquid and more viscous, and producing a shortening and thickening of the muscle as a whole. (*c*) This latter process, on account of the attachment of one end of the muscle to a fixed point and the other end to a lever system, results in the performance of work. The muscle may now relax, and, after the lapse of a suitable period, again undergo shortening, and so on, once every five minutes or so, for about fifteen hours, before markedly showing any sign of the accumulator running down. This feat can be performed in an atmosphere of nitrogen, thus excluding the oxidative removal of the bye-products of the reaction. If one attempted to get not only *maximal* discharges of energy, but tried to get them as quickly as possible, one after the other, the accumulator would show signs of weariness very early—whether it were one of cellulose, lead, acid and water, or one of protein, polysaccharide, lipide, water, etc.

This double process of contraction and relaxation can be carried on quite readily without oxygen, but the next stage, that of recovery—re-charging the storage battery—can only be effected efficiently in the presence of oxygen and circulating water contain-

ing glucose and other crystalloids. The length of time necessary to make the muscle fit to perform again its full complement of work depends, under ideal conditions, on the amount of work it has been called on to do in the immediate past. As indicated above, these are the findings of experiments in which the conditions were not natural.

A muscle doing work in the body is supplied with adequate means (as we shall see in future chapters) of keeping up its store of energy. Like the starting-lighting battery of a motor car, it stores and uses energy simultaneously. It has, moreover, at least one advantage over the car battery: it is self-attending and self-adjusting. It regulates (by means of the balance of hydrophilic colloids and crystalloids) its own water level, and, by the "buffers" in its complex, maintains its H^+ concentration. It also carries out (by means of circulatory changes) its own cleaning and repairs. Further, if it is asked to provide for a heavy discharge fairly regularly, it meets this demand by adding to the size and number of its cells.

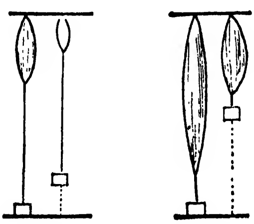

FIG. 41.—Influence of the length of a muscle upon the work done. A muscle one inch long (left-hand figure) in contracting to half its length lifts a weight over half an inch. A muscle of two inches, on the other hand, is capable of lifting the weight over one inch.
(Noël Paton's *Essentials of Human Physiology*.)

This wonderful transformer has been the subject of many investigations—as to its structure and mechanism.

Structure. The units of the accumulator are long cells, which, in muscles like the semi-membranosus and sartorius, are practically as long as the muscle (excluding tendinous attachments). In other muscles (*e.g.* gastrocnemius) they are just a little over half the length of the muscle. These fibres, consisting of fibrils which lie side by side immersed in sarcoplasm, though they, of necessity, all undergo the same amount of shortening together, do not necessarily all develop tension during this process. If the muscle is feebly stimulated only a few fibres actively shorten, the others passively adjusting themselves to keep their due place by their fellows. The stronger the stimulus, the more fibres are involved till, with a certain strength of stimulus, all the fibres are activated. No further increase of stimulus can then produce any further effect. ***That is, other factors remaining constant, the power exhibited by a muscle depends on the number of muscle fibres involved.*** Muscles are, as we have just seen, of different lengths. If a maximal contraction is induced, and each muscle is able to shorten to, say, half its length, then obviously the longer the muscle, the greater will be the distance through which it can pull its load (Fig. 41).

These two factors, number of fibres (thickness) and length of fibres (resting), determine the strength of the muscle. The former gives a measure of the tension developed (cf. volts and number of cells), while the latter bears an obvious relationship to the work which can be done.

$$(1) \qquad \frac{\text{Tension (dynes)} \times \text{length (in cms.)}}{\text{Work (ergs)}} = K_1$$

Isometric and Isotonic Contractions.

It is usual (and simpler) to make the muscle develop tension against a spring so adjusted that the muscle cannot shorten. In an experiment of this sort, the tension developed bears a simple relationship to the heat produced. Such an experiment is called isometric because the length of the muscle is kept equal to its resting length. In a maximal *isometric* contraction Tl/H is a constant ratio (= 5 approx.) applicable practically to all muscles of all animals and over a very wide range of temperatures. Now H, which may be expressed as microcalories, or in work units as ergs, bears a quantitative relationship to one of the metabolites of muscle action, *viz.*, *lactic acid.* Meyerhof expressed this relationship by equating what he called *the isometric coefficient for lactic acid* (K_2) with Tl in this way :

$$K_2 = \frac{\text{Tension (in kgms.)} \times l\,\text{(cms.)}}{\text{lactic acid liberated (mgrms.)}}$$

or in c.g.s. units :

$$(2) \qquad K_2 = \frac{\mathrm{T} \times 1{\cdot}02 \times 10^{-9} \times l}{\text{lactic acid (grams)}}.$$

From these two formulæ one finds that :

(from 1) $\quad Tl = 5H$, and

(from 2) $\quad \mathrm{T}l = \dfrac{K_2}{1{\cdot}02 \times 10^{-9}} \times$ grams of lactic acid.

$$\therefore \qquad \frac{H\text{ (ergs)}}{\text{lactic acid (grams)}} = \frac{K_2}{5 \times 1{\cdot}02 \times 10^{-9}}$$

Substituting the value for $K_2 = 77{\cdot}5$, we find

$$\frac{H}{\text{lactic acid (grams)}} = \frac{77{\cdot}5}{5 \times 1{\cdot}02 \times 10^{-9}} = \frac{1}{65 \times 10^{-12}}.$$

That is, for every erg developed there should be liberated 65×10^{-12} *grams of lactic acid, and, conversely, for every gram of lactic acid appearing one should have* $1{\cdot}5 \times 10^{10}$ *ergs.*

Now when 1 dyne is developed in 1 cm. length of muscle, we have

$$\frac{Tl}{H} = 5, \text{ or } H = \frac{1}{5} \text{ erg.}$$

which should be accompanied by $65 \times 10^{-12}/5 = 13 \times 10^{-12}$ grams of lactic acid, a figure which agrees fairly well with experimental values.

Isotonic Contraction. In the body, of course, muscle naturally *shortens* against a load, thus keeping the tension unaltered. This introduces several fresh factors for consideration. The mechanical act of shortening produces alterations in the thermo-elastic properties of muscle whereby heat is absorbed, while the increase of internal friction resulting from the increased viscosity produces heat. Further, the resistance to movement of the load against which the muscle has to shorten bears a very important quantitative relationship to the heat generated. Fenn has shown that the heat generated in an *isotonic* contraction is greater than the amount generated in an *isometric* contraction by an amount approximately equivalent to the extra work done if the work done is maximal. To take a simple example, in an isometric contraction a muscle evolved 24×10^{-4} calories, while when it did $2{\cdot}2 \times 10^4$ ergs of external work it produced 30×10^{-4} calories. The extra 6×10^{-4} calories are equivalent to $2{\cdot}5 \times 10^4$ ergs, corresponding to the extra external work ($2{\cdot}2 \times 10^4$ ergs) and the internal thermo-elastic and frictional factors.

TABLE XXV

HEAT PRODUCTION UNDER ISOMETRIC AND ISOTONIC CONDITIONS
(Modified from Fenn, *Jour. Physiol.*, LVIII., p. 180)

Mode of Contraction.	Work done (ergs $\times 10^4$).	Heat produced (ergs $\times 10^4$).	Excess Heat (ergs $\times 10^4$).
Isometric .	0	7·3	0
Isotonic .	0·62	8·6	1·3
,,	1·26	9·3	2·0
,,	1·88	10·5	3·2
,,	1·90	11·5	4·2

It will be seen from this Table (XXV.) that, under the conditions of the experiment, the excess heat, measured in ergs, is about twice the value of the external work done.

Muscle causes the conversion of the potential energy of the greater part of the foodstuffs into kinetic energy, *i.e.*, heat and work. The process of conversion is ultimately an oxidative one.

There must, therefore, be a close correlation between the energy evolved and the oxygen intake. If we were to measure the amount of oxygen used by a man resting and kept warm, and then found the extra amount of oxygen he used when he did a measured amount of work, we would find the cost of the work in oxygen. Every gram of glucose requires 1·06 grams of oxygen to convert it into CO_2 and H_2O, and, therefore, we could calculate the cost of the work in glucose from the oxygen consumption, presuming that only glucose was oxidised (Chap. III.). *That is, the basis of muscular activity is oxidation, just as the basis of the activity of a steam engine is the oxidation of coal.*

TABLE XXVI

Gaseous Exchange in *M. Levator Labii Superioris* of the Horse per gram of Muscle per minute (Chauveau and Kaufmann)

	Rest.		Activity.	
	Oxygen absorbed.	CO_2 given out.	Oxygen absorbed.	CO_2 given out.
1	0·0032 c.c.	0·0019 c.c.	0·054 c.c.	0·063 c.c.
2	0·0079	0·0058	0·014	0·018
3	0·0028	0·0026	0·010	0·013

Heat Developed. Every one knows without the use of thermometers or thermopiles that muscular action produces heat. The employment of heat measuring devices applied to isolated muscles or to man as a whole (calorimetric chambers) enables us to formulate equations relating the exact amount of heat generated to the work done and to various other factors. For example, two of the metabolites of muscular action, lactic acid and CO_2, may be equated with either the heat evolved or the tension developed in an isometric contraction or with the work done in an isotonic contraction. As during the cycle of muscular activity, lactic acid disappears at the same time as CO_2 appears, one might consider that the heat developed came from the oxidation of the organic acid. In part, this is the truth, but it is not the whole truth. Let us consider briefly the various stages in the cycle of changes produced when muscle does work.

Muscular Cycle. The muscular cycle from resting state to the return to the resting state is divided into four well-defined stages, *e.g.*, (1) initiation of contraction, (2) maintenance of contraction, (3) relaxation, and (4) recovery or restitution. The three earlier components do not necessarily require the presence of oxygen.

In the recovery stage the anaërobic processes are followed by chemical changes involving the use of oxygen. Practically all the simpler quantitative experiments have been carried out isometrically, as that type of contraction is free from the complication of the various extra factors involved in an isotonic contraction. In the absence of oxygen a muscle produces heat equivalent to $3{\cdot}8 \times 10^7$ ergs for every gram weight of muscle. This amount of heat (0·9 calorie) is divisible over the three anaërobic stages as follows :

1. Initiation of contraction . . $12{\cdot}5 \times 10^6$ ergs.
2. Maintenance of contraction . . $6{\cdot}2 \times 10^6$ ergs.
3. Relaxation . . . . . $11{\cdot}7 \times 10^6$ ergs.

leaving $7{\cdot}6 \times 10^6$ ergs as equivalent to the heat evolved during (4) imperfect anaërobic recovery.

If the contracting muscle has adequate supplies of oxygen, the heat evolved during the last stage will be spread over a considerable period of time and will amount to about six times the anaërobic recovery heat $= 45{\cdot}6 \times 10^6$ ergs. The earlier stages will have the same heat production under both anaërobic and aërobic conditions. During this process of restitution a large amount of lactic acid liberated during the earlier phases disappears (Table XXVII.). Some of it is rebuilt into glycogen absorbing a definite amount of energy which may be produced by the oxidation of other lactic acid molecules. For every gram of lactic acid formed in the earlier stages in the absence of oxygen, about 0·81 gram is restored to muscle to rebuild glycogen, while the remainder, 0·19 gram, is oxidised to CO_2 and H_2O. That is, 0·81 gram lactic acid $\longrightarrow$ 0·81 gram (approx.) of glycogen with absorption of 1008×10^7 ergs $= 240$ cals. 0·19 gram lactic acid $\longrightarrow CO_2 + H_2O + 2873 \times 10^7$ ergs $= 684$ cals.

TABLE XXVII

PERCENTAGE OF GLYCOGEN AND LACTIC ACID IN MUSCLE

MUSCLE.	GLYCOGEN.			LACTIC ACID.		
(Bull frog)	Rest.	Fatigued.	Diff.	Rest.	Fatigued.	Diff.
Gastrocnemius .	0·37	0·16	− 0·21	0·09	0·30	+ 0·21
Thigh . .	0·26	0·07	− 0·19	0·10	0·28	+ 0·18

This table of averages, taken from the experiments of Olmstead and Coulthard (*Amer. Journal of Physiology*, LXXXIV., 1928), shows clearly that what is lost in glycogen content during activity is quantitatively gained in lactic acid content.

The net result, heat evolved less the energy absorbed

$$= 2873 \times 10^7 - 1008 \times 10^7 \text{ ergs}$$
$$= 1865 \times 10^7 \text{ ergs} = 444 \text{ calories.}$$

Determinations of the heat evolved and the lactic acid liberated (Hill and Hartree) show that on isometric contraction 444 calories are evolved during oxidative recovery for every gram of lactic acid. Experiment and calculation agree.

The lactic acid set free in the contraction phase is, in the restitution phase, once more built up into the physico-chemical compound of which it was a part before the arrival of the stimulus provoked a contraction. As A. V. Hill has said, "The lactic acid is part of the machine and not part of the fuel." During contraction it is set free, during restitution it is built up again.

As 0·19 gram out of every gram of glycogen involved disappears during activity, it must be replaced in some way in order to maintain the glycogen content of muscle.

There seems to be little doubt about the experimental evidence regarding the utilisation of glucose during restitution. The glucose stored in the muscle furnishes the main reservoir on which the muscles draw for carrying out this work. There is some evidence, not very clear it is true, suggesting that stored fat may also be called on during muscle restitution. Either because carbohydrate is more readily mobilised or because it requires a lower tension of oxygen for disintegrative oxidation than fat or for both reasons, muscle utilises carbohydrate in preference to fat.

The liberation of lactic acid in the first phase of muscular movement produces not only contraction but a whole series of physico-chemical changes which have got to be reversed during restitution. I. As a dissociable acid (Chap. VII.) it will produce an increase in H ions. II. This increase in hydrion reacts on the colloids in suspension in the muscle, causing them to alter in electrical charge (Chap. VIII.). III. This in turn sets free salts adsorbed to the colloidal surfaces and so produces an increase in osmotic pressure. IV. Further, the membranes will become polarised. V. From III. and IV. will result endosmosis and the water content of muscle will increase.

Roaf has shown that there are definite alterations in H ion concentration associated with different stages of muscle contraction. Macallum proved that activity caused an alteration in the concentration of salts in muscle, and Fletcher has demonstrated the increase in water content after exercise.

What is the effect of temperature on the restitution phase? Theoretically, each of the five sequelæ to the liberation of lactic

acid as mentioned in the preceding paragraph has a positive temperature coefficient. The building up of lactic acid into a complex is accelerated by increase of temperature just like any other chemical reaction.

Rest. During complete inactivity, energy is used for maintaining the muscle in a state of preparedness for action, just as a nation has to spend money maintaining an army in peace time, so the muscle cell must always be ready for action. This is the fifth phase of the muscular cycle—erroneously termed rest. This stage is anything but restful. Just as in peace time the co-ordinating and integrating machine of Empire, the Cabinet, keeps our standing army in a high state of efficiency, so the nervous system constantly sends impulses to the muscles, keeping them ready for instant action. This state of resting readiness may be called the **tone of muscle,** and is, as indicated above, regulated in part by the nervous system (*q.v.*). During rest, energy is expended which if subtracted from the total energy expended during restitution would raise the efficiency of that phase by about 4 per cent.

TABLE XXVIII

OXYGEN USED BY CAT'S *Gastrocnemius M.* (VERZÀR, 1912)

	c.c. Oxygen used by muscle.	
	per minute.	per gram per minute.
Rest (Normal)	0·050	0·003
Contraction	0·178	0·010
Restitution 15 secs. later	0·336	0·020
11 ,, ,,	0·208	0·012
11 ,, ,,	0·154	0·009
Rest (Normal) 146 ,, ,,	0·059	0·0035

Function of Lactic Acid. Lactic acid liberation is the pivot round which all the modern theories of muscular contraction revolve. Five such hypotheses deserve mention.

(1) **Surface Tension Theory.** Lactic acid lowers the surface tension of water and so might alter the interfacial tension of oval elements and their plasma.

A glance at Fig. 42 may make it clear how surface energy may be made to do work.

A wire frame is made, to one side of which is attached a silk thread. Over the whole area is a film of soap. The thread M takes up an indifferent position as shown in (A) as the surface tension at the interface between F and S is exactly balanced by the internal energy of F = internal energy of S. If now the film is broken inside F, say by pricking with a needle, M tends to become a circle. That is, the internal energy of S is increased relatively to that of F. However it is brought about, the result is an increase in the surface tension at the interface $F - S$, *i.e.*, the thread. It is of value to note that it is not necessary for the film to be broken. Theoretically, all that is necessary is a difference in internal energies on the two effective sides of the thread, the lower internal energy being inside the loop. *Further, no matter*

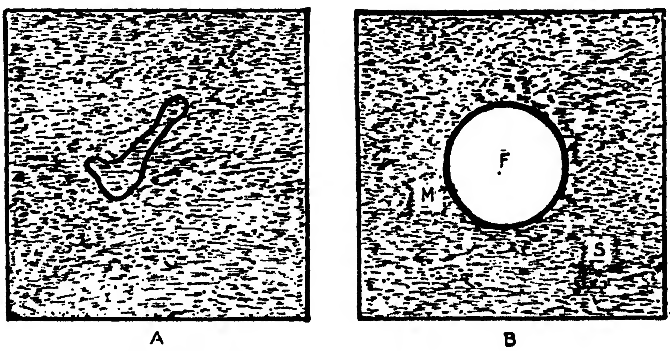

FIG. 42.—Frame of wire enclosing a Soap Film.
In A there is a loop of fine silk floating in the film.
In B the film enclosed by the loop has been broken. (After Van der Mensbrugghe.)

how slight the difference on the two sides of the thread the movement would be maximal—the " all or none " principle.

Muscle consists of a number of chains of long oval elements immersed in sarcoplasm. The membranes (plasmahaut) of the elements may be represented by the thread mentioned above, the protoplasm of element and sarcoplasm as the soap film. Anything which causes a difference of surface tension at the interface between sarcous element and sarcoplasm will cause the element to become spherical, *i.e.* its length would decrease without alteration in volume.

We have seen (Chap. VI.) that a substance cannot produce any effect on a surface unless it can spread over that surface completely. The thinnest layer possible would be one a single molecule deep. Adam has found that the area occupied by a fatty acid molecule forming part of a condensed film on the surface of water is 21×10^{-16} sq. cm. Hill calculates that one molecule of lactic acid should occupy a surface of 24×10^{-16}. Giving the acid the benefit of the

larger value, the total area occupied by the lactic acid liberated when a fibre 1 cm. long develops a dyne would be 24×10^{-16} times the number of molecules. We have seen that under these conditions 13×10^{-12} grams of lactic acid are liberated (p. 170), that is $\frac{13 \times 10^{-12}}{\text{wt of a molecule}}$, approximately 10^{11} molecules. The area coverable would, therefore, be $10^{11} \times 24 \times 10^{-16} = 24 \times 10^{-5}$ cms^2., approx. $\frac{1}{4000}$ of a square centimetre. The muscle fibre being 1 cm. long, its circumference must be $\frac{1}{4000}$ cm. On this edge, a surface tension of 1 dyne has to be produced, *i.e.*, the coefficient of surface tension required would be 4,000 dynes—a perfectly impossible figure. To have a surface effect much more lactic acid is needed. Thus muscular contraction cannot be purely a surface tension effect.

(2) **Osmotic Theory.** The release of lactic acid and other substances of low molecular weight within a membrane known to be semi-permeable in the resting state would produce an endosmosis which might conceivably lead to shortening. Against this theory there is the fact that the muscle does not increase in volume when it thickens and shortens.

(3) **Imbibition Theory.** The increased H^+ concentration produced on stimulation might cause the cell-colloids thus removed further from their isoelectric point to take up more water and swell. This theory suffers from the defects of both the two former theories. There is not enough lactic acid liberated to alter the *p*H of the muscle proteins from the alkaline side of their isoelectric point to the acid side, especially in the presence of electrolytes. (The *p*H of muscle *in situ* is about 7, while the isoelectric point of myoproteins lies between *p*H 4·6 and 5. Myosin is isoelectric at *p*H 3·9.) There is a further objection, namely, it is known that lactic acid is neutralised under ordinary conditions by inorganic bases and to a very small extent (if at all) by proteins. The heat of neutralisation of the acid by proteins is 138 calories and by salts 19 calories per gram of acid. The former value would disturb the energy balance sheet of muscle.

(4) **Liquid Crystal Theory.** Garner and also Clark have suggested that if there is a film of lipoid liquid crystals in or upon the anisotropic bands, then this film might be caused to contract or expand by very slight alterations in *p*H. Clark supposes that the substance in the doubly-refracting bands passes abruptly from a liquid crystal to a solid crystal form under the influence of acid. The solid crystal lattice would have a closer form than the liquid one, *i.e.* shortening would take place. She supports her hypothesis by the production of X-ray diffraction patterns to show the

nature and extent of crystallisation. The X-ray figures, however, do not agree with Garner's. The advantage of this theory—Garner's or Clark's is that relatively large forces are brought into play by an amount of lactic acid which does not need to be sufficient to cover the whole area (see Chap. IX. and Figs. 24 and 26).

These statements about the crystalline structure of elements in muscle are very similar to the modern view of the structure of rubber. Examination of rubber at rest (neither stretched nor pressed) by X-ray interference methods leads one to the conclusion that it is formed of colloidal aggregates of large size. These aggregates consist of highly polymerised rubber swollen by imbibition of rubber not so highly polymerised. In this state the free path of the molecules is limited and they yield no clear interference figure.

If now the rubber is put under stress from any cause (Chap. XVII.) the liquid phase is expelled from the aggregates to the continuous phase. Clear interference figures—crystal like—are produced. When released, the substance tends to regain its state of unstressed equilibrium and the crystal structure disappears. The incorporation of a straight-chain fatty acid in the rubber leads to a great increase in tensile strength, increasing with the length of the carbon chain up to 14 carbon atoms, and giving marked X-ray interference figures when put under torsion, stretch or compression.

(5) **Condenser Theory.** Hill (1925) brought forward a new conception, viz., that the fibrils, of which there are somewhere about 100 per fibre, are little negatively-charged cylinders of protein, surrounded by a cloud of attendant electrons, the whole constituting a tiny condenser. "Such a condenser would be in a state of strain under the mutual repulsion of the elements of charge occupying its plates. The sudden liberation of lactic acid in the neighbourhood of the negatively-charged protein surface would cause a discharge of the condenser by the formation of sodium lactate and ionised protein. The mutual repulsion of the charges would then be obliterated and the condenser would tend to shorten. The force developed in such a condenser suddenly discharged can be calculated, provided we know its dimensions and the density of its charge." Hill calculates that a monomolecular film deposited on the surface of the condenser could easily account for the liberation of a force of 5,000 dynes per cm. edge (cf. Fig. 14).

The temperature co-efficient for a complete muscle cycle is 1·8, which means that the rate of the physico-chemical reactions involved is almost doubled by an increase of 10° C. As we have seen this rate is a compromise between the decrease in the

rate of the physical reactions of the contraction phases and the increases in the physical and chemical reactions of the restitution phase.

Efficiency of a Contraction. The mechanical efficiency of a contraction is the fraction of the total energy expended which can be recovered as external work.

It is found from the formula

$$E_g = \frac{a}{b} \times 100,$$

where a = actual work done (in cals.) per unit of time.
b = total energy used (in cals.) ,, ,, ,,

This gives the gross efficiency. The net efficiency is obtained by correcting for the energy expended during complete inactivity during a similar unit of time.

Net efficiency $$E_n = \frac{a}{b - c} \times 100,$$

where c = energy expended during inactivity (in cals.) per unit of time.

As no external work is done during inactivity it is difficult to assess the value of the efficiency of this phase.

The values obtained for a complete muscular cycle (contraction and recovery) vary somewhat with the animal chosen and with various other factors, such as temperature, rate of work, load, etc. So far, only the efficiency of individual muscles acting isometrically (or of the entire muscular machine (Chap. XXXVIII.)) have been estimated. The maximum gross efficiency is under 50 per cent. Various attempts have been made to apply findings from experiments on isolated muscles to muscles *in situ*. For example, the *biceps brachialis* of man pulling against an immovable object for about 1·4 seconds, turns 26 per cent. of the available energy into realisable work and 74 per cent. to domestic purposes and to warming the muscle. If the duration of the contraction is greater or less than 1·4 seconds the efficiency of the biceps falls off. Similarly the optimum load, the optimum rate of contraction, as well as the optimum duration of the contraction, could be found for any muscle or group of muscles (see Chap. XXXVIII.).

Training. The regular use of a muscle or group of muscles in a certain way leads to their more efficient use. Repetition not only causes a " warming up " of the muscle, but leads to a decreased shortening viscosity (cf. Expt. 31 (b), p. 531). Other factors, cardiac and nervous, enter into the question of the effectiveness of training as applied to a complete animal.

Character of the Electrical Variations in Muscle.

The existence of electrical currents in tissues did not find direct proof until the year 1824, when Nobili devised the galvanometer and showed that "natural currents" occur in the frog. Other investigators examined this current of rest and found that in a muscle removed from the body the current *in the muscle* passed from the ends to the middle and in the *external* (*galvanometer*) *circuit* from the middle to the ends. It has been conclusively proved that these "natural" currents are not natural at all but are an indication of injury to the muscle. Slight injury is unavoidable in the process of removing the muscle from the body--the less the preparation is injured the smaller is the current obtained from it. In other words, *normal resting muscle is isoelectric and therefore currentless* (Fig. 38).

Current of Injury or **Demarcation Current.**

The injured part of a muscle is like the injured part of any cell (p. 152 and Fig. 38), "zincative" or electropositive to the uninjured part. That is, if non-polarisable electrodes are led off from injured and non-injured parts to a current-detecting device, it will indicate a passage of current from the **uninjured to the injured** parts of the preparation *through* the galvanometer. *Within the tissue*, of course, the circuit will be completed by the passage of the current from injured to uninjured. This difference of electromotive force may be demonstrated without a galvanometer. If the nerve from an uninjured muscle be laid over an injured muscle in such a way that at one point it touches a cut portion, then, the undamaged muscle will contract every time the circuit is completed by laying a second point of the nerve on an uninjured portion of the injured muscle.

This difference in E.M.F. persists as long as the injury. In a degenerating muscle its degenerating portion is electropositive, galvanometrically negative or "zincative" to its normal portion. Naturally, the difference ceases when degeneration is complete. The whole mass is then isoelectric. The current is due, as has previously been explained (p. 153), to physico-chemical differences at the junction of living and *dying* tissue. Dead tissue gives no current.

Current of Action (Fig. 43).

Similar physico-chemical changes are answerable for the wave of "negativity" which precedes the mechanical change in a contracting muscle. The part which is just about to contract is electropositive, or "zincative," to the rest. Consider for a

moment a muscle, say 5 cm. long. The preparation is supposed to be perfect and, therefore, there will be no demarcation current. If such a muscle be stimulated by a single induction shock at one end and two points **A** 3 cm. and **B** 5 cm. from the point of stimulation be led to an electrometer, then each stimulus will cause a wave of contraction to pass along the muscle, preceded by a wave of "negativity." That is, **A** will become "zincative" to the rest of the muscle—so that current would pass through a galvanometer from **B** to **A** (Fig. 43 (*a*)). A fraction of a second later, the disturbance will have passed on to **B** which will now be "zincative" to the rest, causing a current to pass through the galvanometer from **A** to **B** (Fig. 43 (*b*)). That is, **A** has first been

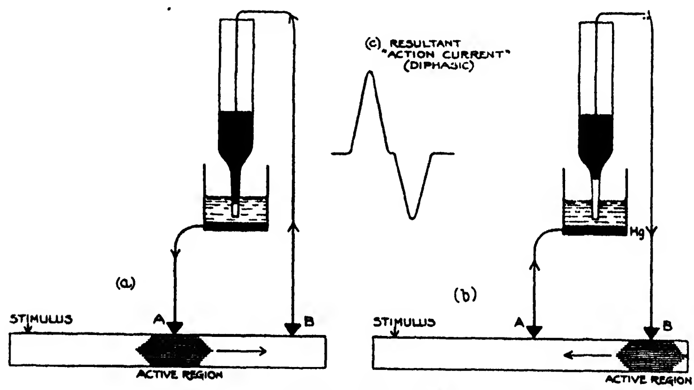

FIG. 43.—Diagram to show the diphasic character of the current of action in muscle. (See text for explanation.) Fig. *c* (in centre) is a representation of the photographic trace obtained by protecting the shadow of the mercury in the capillary tube through a lens on to a rotating strip of sensitised paper. See Fig. 92.

electropositive and then electronegative to **B**. Such a current is termed **diphasic** and is an indication of a propagated change (Fig. 43 (*c*)).

A muscle nerve preparation may be used to demonstrate the presence of the current of action. If the sciatic nerve of a frog's gastrocnemius be placed on another gastrocnemius, the former muscle may be made to contract by stimulating the nerve of the latter. The essential point about this preparation which is called the **rheoscopic frog** is that it actually proves the occurrence of a diphasic current in muscle in consequence of its activity. If the free nerve is stimulated by a tetanising current both muscles go into tetanus. This **secondary tetanus** demonstrates that although the stimuli are being applied so rapidly that the contractions of the "battery" muscle are fused, the diphasic nature of the excitatory process is still quite distinct and is indicated by the contraction of the "galvanometer" muscle.

The current of action may be considered as inclusive of the current of injury. Injury is stimulation, or, conversely, stimulation is a temporary injury. Therefore, the current produced by an injury confined to a small area should be weaker than that obtained by the excitation of the whole muscle. The E.M.F. of the current of action of a sartorius is about 0·085 volts, while the demarcation current may be about 0·053 volts. The diphasic current of action is of short duration, while the monophasic current of injury continues as long as the muscle lives in an injured condition.

Further Reading

Hill. "Living Machinery." Bell.
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CHAPTER XV

MANUFACTURING CELLS

"The extreme assumption that the laws of Physics and Chemistry are inadequate to explain the causation of vital phenomena is, of course, not justifiable, for it postulates that we fully comprehend now all the laws of the physical world."

MACALLUM.

In the preceding chapter attention has been drawn to the muscles as cell communities which consume power but do not produce commodities for the use of the body as a whole. Other cell groups, the glands, may be regarded as industrial communities manufacturing goods for use elsewhere. Others again are mere handlers of goods. These latter, the organs of absorption and of excretion (negative absorption), do not as a rule alter the chemical state of the material, raw or manufactured, that they handle. They accept delivery, *repack in suitable containers* it may be, and forward for transport.

The secretory glands may be divided for convenience into two classes. First, those which by means of a duct, opening *outside* the body, secrete manufactured material. The glands of the alimentary tract and the skin glands (sweat and milk) belong to this class. The other class prepares material which is of value to other cells in the body. They secrete into the blood stream. The former may be termed organs of external secretion, or exocrine glands. The latter are called organs of internal secretion, ductless glands or endocrine organs.

As far as is known the principle underlying the activities of all glands is the same. Each manufactures some material which is stored up, and when wanted, this material is washed out by a stream of water. That is, they all consist of a workshop and a dispatch department. These two functions are seemingly under different control and have to be studied separately.

The work done by a gland may be divided into phases—just as we saw that muscle work could be so treated—viz.: (*a*) Activity, (*b*) Restitution, (*c*) Rest or Maintenance.

(*a*) **Activity.** The outsider may gauge the activity of a factory by studying its output, and so, much may be learned of a gland by noting how much it secretes and when. Some glands secrete continuously, others in spurts. With the former, should be

placed the endocrine organs, with the latter, the digestive glands. Of course those which maintain a steady output may, under stress, greatly accelerate their rate of secretion, and of the latter class the salivary glands at least maintain normally a level of secretion which under a suitable stimulus is enormously increased.

There seems no doubt but that the industrial cell-group consists of four different parts corresponding to their activities. (I.) The factory itself where the secretion is prepared. (II.) The store room where it is packed and kept in bulk. (III.) The dispatch department where it is first packed small and ready for delivery and then (IV.) the actual delivery department. Generally when we speak of the activity of a gland we refer exclusively to this last function, viz., active secretion. What then regulates the rate of secretion? The same factors come into play which operate in our industrial world, viz.:

1. Stock on hand.
2. Rate of output from workshop.
3. Efficiency of the dispatchers.
4. Demand for goods.

Normally, the store of goods on hand and the rate of manufacture do not materially influence the output. Of course, if the operatives are poorly nourished or badly supplied with raw material, then output will fall. Under certain pathological conditions, a state of temporary or chronic over-production occurs. Similarly, insufficiently fed or overworked dispatchers will perform their duties half-heartedly and output will be decreased, but as a rule this factor does not come into play.

The decisive element controlling rate of output is the demand for goods. The store of goods is drawn upon and the factory speeds up to replenish the store. If the stored material is sent out more rapidly than it can be replaced, then overtime has to be worked in the factory, and if persisted in, industrial fatigue is caused and total cessation of work is the final result (cf. Secretion of Milk).

These various conditions may be studied conveniently by studying the intake of oxygen or output of CO_2. In some instances the intake of potential energy may be measured. From these it is found, as in muscle, that a very small proportion of the total O_2 intake goes to the dispatch department. That is, the actual setting free of the secretion does not require much energy.

(*b*) **Restitution.** Just as in muscular, so in glandular activity the great proportion of the oxygen used is associated with the phase of restitution. Energy is required for the building up of material to replace that lost during secretion.

(*c*) Then, as in muscle, the gland requires a certain amount of energy for domestic use. To keep its parts in repair and to preserve its identity, it requires a maintenance allowance. The following figure from Barcroft will help the student to realise the energy expended during these three phases in the activity of the salivary gland in the cat.

From the Figure (44) it will be seen that the maximal rate of secretion occurs some time before the maximal consumption of oxygen, and that the increased consumption of oxygen lasts for some time after the saliva has ceased to flow. Barcroft and his colleagues found that the length of this period of increased oxygen consumption depended upon the previous degree of activity of the gland as well as on its functional capacity. In other words, if a previous inroad upon the store of material had not been made

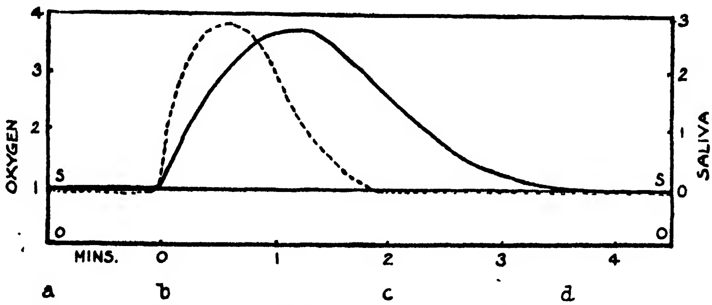


FIG. 44.—Oxygen used by the salivary gland during rest, activity and restitution. From *a* to *b*, the gland was not secreting, but was using a fairly constant amount of oxygen. From *b* to *c*, the gland was active—secreting saliva at the rate denoted by the dotted line. From *c* to *d*, the gland was being restored to its pre-secretory state. *o-o* = oxygen base line. The area, *ooss*, represents the basal or resting metabolism of the gland. Dark continuous line = oxygen consumption. *s-s* = base line for saliva. Dotted line = saliva formed in c.c. per minute. (After Barcroft.)

up, the factory cells would have to work at high pressure to keep pace with the demand. Work at high pressure is not economical. Each gram of secreted material is formed at an increased cost in oxygen and energy.

The energy required for secretion comes from the oxidation of glucose. (Again compare with muscle.) For the dispatch of the material, little extra oxygen and little extra glucose is required. Asher and Karalov found that the restitution phase required the most energy. That is, the glucose content of the blood was markedly diminished in the post-secretory period. The amount of glucose used is parallel to the oxygen consumption, as one would expect.

The mechanism of secretion has been provocative of much controversy. A regular pitched battle results when vitalists, neovitalists and mechanists discuss the problem. What are the facts ?

1. Microscopical examination of the gland shows that during inactivity the lumen (storehouse) becomes filled with granules and the gland increases in volume. When the gland is excited to secretion, these granules disappear with the secreted fluid and the gland decreases in volume.

2. Water passes from the blood into the gland and out with the secretion.

There seems to be no difficulty in giving a plausible explanation of the second of these phenomena. The postulation of a semi-permeable membrane is sufficient. The first fact presents difficulties.

(*a*) The osmotic pressure of the secretion is often greater than the osmotic pressure of the blood.

(*b*) The pressure in the duct against which the saliva may be secreted was found by Ludwig, in 1851, to be greater than that of the artery supplying the gland. Hill and Flack found that the pressure of secretion was as high as 240 mm. Hg. with an arterial pressure of 130 mm.

3. Macallum demonstrated alterations in surface tension during secretory activity.

As mentioned in Chap. VI., this worker made use of the Gibbs-Thompson distribution of salts to determine the relative values of surface tension in cells which had been killed and fixed almost instantaneously. Theoretically, in an active gland there must be at least three different values for surface tension, viz. :

(1) Cell-lymph interface, *i.e.*, on the outer face through which raw material and power enter.

(2) Cell-cell interface where the cell wall is in contact with some of the other cells of the gland.

(3) Cell-lumen interface through which the secretion and the leaching water pass.

He found that, during activity, there was the densest condensation of potassium at (3), the cell-lumen interface, less potassium was found at the cell-cell interface and least at (1), the cell-lymph interface, while when the gland was at rest there was no marked difference between the interfaces. According to the Gibbs-Thompson principle these results may be taken as an indication—

(*a*) That during rest there is no marked difference of surface tension at the gland interfaces, and

(*b*) That during activity a high tension develops at the surface between cell and lymph, a low tension between cell and lumen and that the cell-cell interface has a value intermediate.

4. **Blood Supply.** It is well known that during glandular activity

there is an increase in the rate at which blood enters the gland. In other words, raw material and power are taken into the factory at an increased rate. The view was at one time held that the secretion was due to this increased flow of blood. Barcroft's experiments have shown that this cannot be true, because

(*a*) The increase in the blood flow through the organ is initiated after the secretion begins and is continued for some time after secretion has ceased ; and

(*b*) Vaso-dilatation may take place without secretion.

The increase in blood flow or vasodilatation is a consequence of secretion and not the secretion a result of the vasodilatation. The actively secreting gland, as it were, sends out a call for oxygen, for power and for material. This call is in part met by this increase in the transport service (see Chap. XXV.).

5. **Electric Potential.** Alterations take place in electrical potential of one part of the gland to another. These have been studied principally by Bayliss and Bradford on the salivary gland and by Orbelli on the skin glands of the frog. The results vary somewhat with the means of investigation, but may be taken as indicating two things.

(*a*) The secretion of water, *i.e.*, dispatch of secretion, is a different function of the gland or a function of a different mechanism in the gland from the elaboration of the true secretory material. That is, we have to consider two phenomena, the preparation of material and its flooding out of the cell by water. The latter is accompanied by—

(*b*) A large difference of potential between the cell-lymph interface and the cell-lumen interface, the former by a small potential difference of the opposite sign from the latter.

The cause of the larger difference may be sought in the increased permeability (lowered surface tension) of the cell-lumen interface ; allowing free passage to cat-ion and an-ion. That is, at this surface the electrical potential recorded will be that of the interior of the cell (cf. injured muscle).

An explanation of the potential difference developed during the elaboration of secretion is more difficult. There seems no doubt that just before being carried out through the duct, the granules undergo some change. The large colloidal particles either break down into smaller particles or go into solution. Either of these actions is accompanied by the setting free of adsorbed salts and alterations in the electrical charge.

6. These two processes, water secretion and the elaboration of the organic secretory material, seem to be controlled by different sets of nerves. Secretory nerves when stimulated cause the

gland to be flooded with water, while "trophic" nerve fibres have to do with the emission of the granular material of the secretion. (Heidenhain, 1868, and Babkin, 1913.) Both sets of fibres may go to the gland in the same nerve. It is interesting to note that acid and other irritants excite secretory fibres only, while normal excitants stimulate both secretory and trophic fibres.

Can we, from these facts, construct a picture of the mechanism of secretion ?

(1) The formation of granules in the cell may be similar to the formation of starch in the plant. Substances are thus put out of action. The colloidal granules not only have a low osmotic pressure but they adsorb crystalloids and so prevent endosmosis.

(2) On stimulation, these granules are broken down into smaller particles and water rushes in. It may be that stimulation of the gland follows the same course as in muscle and produces acid. This acid would interfere (*a*) with the colloids present, especially with their power to hold water (imbibition) and salts (adsorption) and so bring about alterations in their size, electrical charge and the osmotic pressure of their dispersion medium. (*b*) Acid has a direct action on the electrical charge of any solution and, therefore, acts on its surface tension, lowering it. The only surface where this can take place is between the cell and the lumen, because the alkaline reserve of the blood is sufficient to keep the cell-lymph interface normal or rather supernormal, while the cell-cell interfaces obviously need not be considered.

In short, the arrival of the *appropriate* stimulus causes the cell to draw on its store of material, alter the packing of the material and launch it into the duct on a current of water. The stimulus may be nervous or it may be a hormone (chemical messenger) formed in another organ and transported to the gland by the blood.

Bayliss and Hill have shown that the salivary gland does not become heated during activity. From this we may deduce that all the additional energy set free during the course of activity is used in doing work (in elaborating the secretion and in setting it free, etc.), and in maintaining to some extent the normal temperature of the body (cf. Muscle). Thus leaving alone the latter sink of energy we may assume that a gland is 100 per cent. efficient, and calculate the work done from the oxygen intake or carbon dioxide output or from the diminution of sugar in the blood passing through it.

Of the mechanism of those glands which secrete directly into the blood stream little is known. Seemingly, the secretion is

extruded from the cell and washed away by blood, lymph or cerebro-spinal fluid. Their oxygen consumption has never been measured nor yet their utilisation of glucose.

Further Reading

Swale Vincent. "An Introduction to the Study of Secretion." Arnold.

CHAPTER XVI

THE ARMY FOR HOME DEFENCE

"Whatever uncertainty and variety may appear in the world, we remark, nevertheless, a certain secret concatenation and regular order at all times carried on by Providence, which causes everything to proceed in its course, and to follow the law of its destiny." LA ROCHEFOUCAULD.

THERE are certain cells and certain cell-communities whose function it is to guard the organism from the invasion of its cells by noxious substances and by predatory parasites.

1. **Ciliated Epithelial Cells.** In certain parts of the body, where it might be possible for solid matter in a fine state of division to find its way into hollow visci, a peculiar type of protective mechanism is found. The entrance to the cavity is lined by a layer of more or less columnar cells on the exposed surface of each of which is a bunch of fine tapering filaments. During life the cilia move in such a way as to produce a flow outwards of the fluid bathing them. Ciliated epithelium is found lining the whole extent of the air-passages (except upper part of nares, lower part of pharynx, terminal bronchioles and pulmonary alveoli, *q.v.*). It occurs also in the uterus and uterine tubes ; in the efferent tubes of the testes. [As we shall learn later, ciliated epithelium is found in other places, *e.g.*, central canal of spinal medulla and ventricles of the brain where it provokes movement of the cerebro-spinal fluid.]

There are two phases in the movement of any cilium, (*a*) a rapid stroke in the direction in which the action is to be effective, and (*b*) a slow return stroke. Further, although all the cilia attached to any one cell or row of cells act synchronously, there is a metachronical rhythm about the whole ciliated surface, *i.e.*, any individual cell begins its effective stroke slightly later than the cell immediately internal to it and slightly earlier than the cell lying external to it. In this way a progressive wave motion is produced, carrying towards the exterior the extraneous matter, dust, etc., entangled in the mucus deposited on the ciliated surface from numerous glands.

In considering the efficiency of this means of protection, one has to take into account the amplitude of the strokes, the frequency of the strokes of the cilia as a whole, and the exactitude of the timing of their concerted rhythm. Human cilia are not very long,

and we have no means of measuring the amplitude of their beat. The frequency is somewhere about 10 per second. One may say that the energy necessary for an effective stroke would be directly proportional to the amplitude and to the square of the velocity. $E = k.sv^2$ (where k is a constant, s the amplitude, and v the velocity of stroke). Therefore the work done in an effective (unloaded) stroke would be ksv^2/t, where t is time, or as

$$\frac{\dot{s}}{t} = \frac{1}{v}$$

$$W = kv^3.$$

The value of W varies from almost 0 to probably just under 30 ergs. The activity of the cilia is modified by almost any factor that modifies protoplasmic activity. The relative concentrations of oxygen, carbon dioxide, hydrogen ion and various salts are factors any alteration of which will cause a change in activity. Increase of temperature up to a critical value causes increased activity. Especially interesting is the alteration produced in rhythm and rate by loading the cilia. The placing of an inert powder on the mucus-covered tips is sufficient to excite active flagellation.

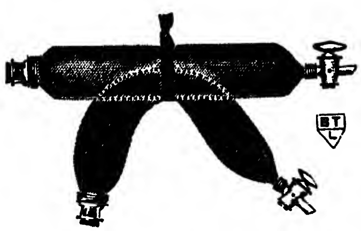


FIG. 45.—To demonstrate turgor. The sausage-shaped membrane filled with a sugar solution is flaccid. When immersed in water, endosmosis occurs and the vessel becomes rigid.

We have no evidence as to the mechanism involved in the production of this movement. Schafer supposes an increase in liquid pressure in a hollow blind tube (cf. Fig. 45 and Expt. 6 Part II.,), but gives no explanation as to how this alternate flow and ebb of pressure into the cilia is produced.

2. **Reticulo-endothelial System.** It has been found that when a substance like the dye, lithium carmine, a colloid, is injected into the blood stream of a living animal, it is not immediately excreted nor is it uniformly distributed throughout the tissues. Muscle fibres, ganglion and glial cells appear to take up none of the dye, while (in the cat) about two-fifths is found in the liver, half in the lungs, one-twentieth in the spleen, and the remainder in large part in the kidney, lympoid tissue, and certain cells moving freely in the blood stream, the true vascular endothelium being stained very feebly. If, instead of killing the animal for examination an hour after the injection, the animal were allowed to live for about twelve hours, then most of the dye would be found in certain cells of the liver. The cells which are capable of ingesting colloidal particles

(suspensoids or emulsoids) have been grouped together under the name of the reticulo-endothelial system. They are found in the lungs, spleen, liver, bone-marrow, lympoid tissue generally, and in the blood.

Function. They have a special avidity for acid suspensions (colloids, small particulate suspensions, fat-dust, cholesterol, bacteria and fragmented and moribund erythrocytes). Those that are free in the blood stream, *e.g.*, monocytes (histiocytes) and endotheliocytes, are electrically charged bodies at the hydrogen ion concentration of the blood (pH 7·4) and move to the cathode in an electric field. The bacteria in a suspension of *B. typhosus* move to the anode. When the smaller electro-negative colloidal mass (bacterium) comes within the sphere of influence of the larger oppositely-charged mass (histiocyte) the result is adsorption and ultimately absorption. The processes whereby bacteria are engulfed are hastened by a gathering together of the bacteria into clumps which may be absorbed as such. The large cell thus disposes of a larger number of the smaller cells at each encounter. Agglutination (the clumping of bacteria) bears a general resemblance to the process of sensitisation of hydrophilic colloids. In this connection it is interesting to note that the salts of the plasma are essential for the process. If, by means of dialysis, they are reduced appreciably, or if their balance is markedly disturbed agglutination does not take place, and thus bacterial absorption remains a slow process.

It has been mentioned above that the cells of the reticulo-endothelial system show a special preference for acidic dyes, *e.g.*, *trypan blue* or pyrrol blue, and thus have become known as "pyrrol" cells. Compare with this the staining action (adsorption) of these dyes on colloids (p. 95). Solid particles may also be taken up by these cells. The special endothelial cells of the lung may be found full of carbon particles, silica powder, asbestos dust, or finely divided metal in animals to which these substances have been administered either by insufflation or by injection (cf. protection of suspensoids by hydrophilic colloids, p. 93).

Substances of a fatty nature or substances soluble in fats tend to collect, under certain conditions, in reticulo-endothelial cells. The esters of the monohydric alcohol, cholesterol, may, in this way, fill the reticular cells of the spleen to such an extent that that organ undergoes enlargement. We can again, as a first approximation, suggest that lipide and reticulo-endothelial cells differ somewhat in electrical charge and thus tend to come more closely together. The lipide may then be oriented in the first instance, so that its polar group is in the plasma and its fatty portion in the

lipide membrane surrounding the specialised cell (p. 51). As the major length of the cholesterol molecule is fat soluble, the tendency will be for the OH group to be pulled close to the surface of the cell. In this position the hydrophilic cell-substance will enter into competition with the surrounding plasma for the polar group. The balance between *Ca* and *K* plays a very important part in this tug-of-war. If the blood *Ca* is on the low side and the cholesterol high (*e.g.*, in starvation or diabetes) the cell contents win, and the cholesterol and other fatty bodies are tucked away in the cell. On the other hand, when the calcium is high (or *K*

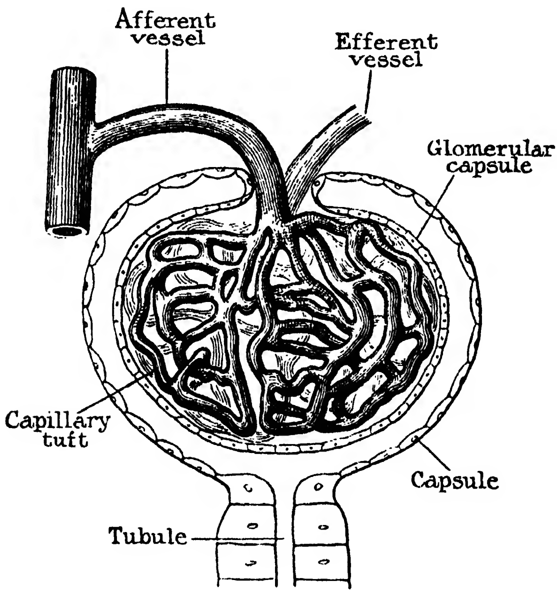


FIG. 46.—Diagram of Malpighian corpuscle. (From Cushny's *Secretion of Urine*.)

low) the tendency is for the cholesterol to form aggregates with some calcium soap as nuclei (p. 107) and gall-stones may be formed.

One of the most important functions of the reticulo-endothelial system is hæmolyto-poietic. This is especially developed in bone marrow, spleen, liver, and lymph nodes. These organs are rich in sessile reticulo-endothelial cells, having the *rôle* (in bone marrow) of forming or of destroying erythrocytes, and in the others of destroying them (*q.v.*).

Ultimately the ingested particles or their derivatives are carried by wandering cells to the liver and excreted with the bile into the gut. That is, the heavy metals, the colloidal dyes and disintegrated blood pigment are eliminated with the fæces (*q.v.*).

3. **The kidneys** are the great eliminating organs of the body. Each of them is built up of a number of long unbranched tubes closed at one end and, at the other, opening, along with several other similar tubules, into a common collecting tubule. This in turn opens into the pelvis of the kidney. The production of urine goes on in these unbranched tubules, the collecting tubule serving apparently only as a conduit to the pelvis. The closed end of each tubule is invaginated within itself to form a **Bowman's capsule,** where its epithelium lies in close contact with the capillary tuft of blood vessels—the whole end-structure being called a **Malpighian corpuscle** (Fig. 46).

The kidney does not manufacture any of the constituents of its secretion except hippuric acid and probably a small quantity of urea, but merely eliminates unchanged certain of the bodies brought to it by the blood.

It is not a mere filter, as the concentration of the constituents of the urine are vastly different from the concentrations of the same substances in the blood. There seems to be a threshold value for each and every substance in the blood. That is, whenever any substance capable of elimination by the kidney oversteps its threshold value, it is, in general, excreted till the excess has been removed. In this way, the kidney acts as a regulator of the water and salt content of the system. Abnormal constituents of the blood, except those entangled in the reticulo-endothelial cells and excreted *viâ* bile and fæces, pass in their entirety into the urine. Not only is there an alteration in the relative concentrations of the various substances eliminated, but there is in general an increase in the concentration of solutes. This process of concentration necessitates the expenditure of energy.

It is very difficult to get reliable experimental results from the kidney. Its nature, blood supply and position do not lend themselves to surgical interference, and the student ought to be keenly critical of results which are produced under uncontrolled abnormal conditions. Some facts, however, are obtainable and may be detailed here shortly.

1. **Function.** No one doubts that the kidney as a cell-community has specialised in excretion. Every cell in the body has the power of eliminating waste products. Most of these substances find their way into the blood and most of those that are non-volatile are voided by the kidney cells.

2. **Structure.** The functioning parts seem to be structurally, two, (*a*) the capsules, and (*b*) the tubules. Each capsule is lined by flattened pavement cells supported by a delicate basement membrane. A tubule passes from each capsule, joins with other

tubules, and finally opens into the pelvis of the kidney. As the structure of the kidney is intimately related to its function we must briefly follow the course of a tubule from capsule to ureter and note the type of epithelium with which it is lined.

Capsule, flat thin endothelial cells; ⟶ neck of tubule, cylindrical epithelial cells; ⟶ first convoluted tubule, columnar epithelium, indefinite outlines, rows of granules arranged vertically to base of cells, striated free border; ⟶ U-shaped loop of Henle, descending limb has flattened epithelium while ascending limb is similar to convoluted tubules, but with less striation; ⟶ second convoluted tubule, as first; ⟶ junctional tubule, cubical or columnar cells with no granules; ⟶ straight collecting tubule, same as junctional.

3. **Blood Supply.** The artery supplying the kidney breaks up in the cortex into a large number of arterioles, each of which forms a nodule or glomerulus invaginated in Bowman's capsule. The capillaries again coalesce to form the efferent vessel, and this again breaks into a number of capillaries entwining round the tubules. After this the blood leaves the kidney by way of the renal vein. That is, the blood is first supplied to the glomeruli and then to the tubules. In the mammal, the capillaries surrounding the tubules *may* receive some blood which has not passed through the glomeruli.

4. **Blood Pressure and Secretion.** If the blood pressure is lowered to between 40 and 30 mm. Hg secretion stops. Starling measured the osmotic pressure of plasma and found it to be about 30 mm. Hg. It is generally inferred from this that unless the blood pressure be greater than the osmotic pressure of the plasma colloids no secretion will take place. Starling confirmed this by obstructing the ureter so that the hydrostatic pressure therein was equal to 92 mm. Hg when secretion stopped. The blood pressure was 133. That gives a filtration pressure of 133 minus the osmotic pull back of the colloids (30), *i.e.* 103, approximately equal to the pressure in the ureter. (See Chap. XXII., O.P. of Plasma.)

Function of Capsule. It is only fair to point out that, though modern theorists are at one regarding the forced filtration of colloid-free blood through the capsule by means of glomerular pressure, *i.e.* heart work, arguments against the supposition may be found. For instance, the thin layer of epithelial cells is not strengthened in any way to stand a large filtration pressure. Again it is doubtful whether any such pressure exists in the glomeruli. Measurements are given of general arterial pressure, say in the carotid artery. The capillary pressure may be under one-fifth of this.

Simple diffusion through a membrane impermeable to colloids will answer as well. Increase in blood flow instead of pressure regulates the amount of dialysate. Furthermore, it is generally stated that capsular fluid has the same composition as blood, less the colloids. No direct evidence of this has been produced. Theoretically, it is not probable. Colloids have not only the power of holding salts by adsorption, but globulin especially, holds water and sodium chloride in solution. This was proved by Milroy and Donegan, who showed that even when 250 c.c. of water per hour passed the outside of a collodion membrane, a solution of globulin in 0·15 per cent. sodium chloride lost practically no salt in six hours. Any salt over the amount mentioned rapidly passed through the membrane.

It does not matter whether the filtration or the dialysing function of the capsule is accepted as correct, it is clear that no energy is expended by the kidney in carrying out the process. If filtration occurs, the energy to force the fluid through the filter comes from the heart; if dialysis be the process, molecular forces are involved. Proof of this lack of work on the part of the capsular cells may be sought in the oxygen consumption of the kidney when it is known that the tubule cells are not particularly active. Such is the case when a diuresis (free flow of urine) is caused by the injection of Ringer's solution. This solution contains the various salts of the blood plasma in their correct proportions, and thus its administration leads to a temporary dilution of the colloids of the plasma. The dilution is merely temporary, because there is an almost immediate increase in the amount of urine secreted, but the increase in the oxygen consumption of the kidney is relatively small (Fig. 47).

Saline Diuresis. The introduction of the saline fluid has caused:

(1) A temporary increase in the volume of blood corresponding to the amount of the fluid injected.

(2) An increase in general blood pressure and therefore an increased pressure in the renal arterioles.

(3) An increase in the rate of the blood flow through the kidney vessels. Richards and Schmidt showed that ordinarily, blood flows through only a part of the glomerular capillaries, but that saline diuretics cause the closed capillaries to open, and so allow the blood to flow through a greater number.

(4) A decrease in the concentration of the corpuscles of the blood. This results in a decreased oxygen carrying power and a decreased viscosity.

(5) A dilution of the colloids of the blood.

The saline diuresis may have been caused by all or any of these concomitants. They may be eliminated one by one.

(1, 2, and 3) Increased blood volume, pressure and flow may be considered together. Increase in pressure, etc., produced mechanically without altering the concentration of corpuscles or of colloids, certainly does produce an increased flow of urine, the constituents of which have a concentration approximating to that produced after injection of Ringer's solution. Barcroft and Straube overcame this difficulty very ingeniously.

They previously removed a quantity of blood equal in volume to the Ringer they were about to inject, thus keeping the blood volume, etc., normal. The diuresis was produced as before, entailing no extra oxygen consumption.

(4) The addition of blood corpuscles to make up the deficient concentration made no appreciable difference in the flow of urine.

(5) Knowlton introduced a colloid, gum acacia or gelatine into the perfusion fluid so that the colloidal osmotic pressure of the injected fluid was equal to that of the blood (25–30 mm. of Hg). This prevented the onset of marked diuresis, gelatine being more efficient in this respect than gum acacia. Two causes may be ascribed to the lower efficiency of gum: (*a*) its lower osmotic pressure (5 per cent. gum has an osmotic pressure of about 12 mm. compared with 5 per cent. gelatine whose osmotic pressure is 23 mm. of Hg. Bayliss recommends a 7 per cent. solution of gum). (*b*) Gelatine has a certain water-holding power which is altered by treatment with salts (see Imbibition, Chap. VIII.).

The conclusion that one would draw from this series of experiments is that the passage of fluid and salts through the kidney by filtration or dialysis is controlled by the concentration of colloids in the blood plasma.

Sulphate diuresis. Let us consider now a case where oxygen is consumed and, presumably, work done. Sodium sulphate is a diuretic, *i.e.*, causes a free flow of urine. It is less diffusible than sodium chloride, and may be retained by a collodion membrane which will allow the chloride to pass through. Yet in the kidney, the very reverse seems to take place. Sodium chloride acts very much like Ringer's solution, and though after a large injection of NaCl solution the chloride content of the urine rises, it does not materially alter the concentration of the other solutes. On the other hand, the injection of Na_2SO_4 is followed by the secretion of a urine almost entirely an aqueous solution of the sulphate. After the injection of a solution containing equal amounts of chloride and sulphate of sodium, more sulphate than chloride is excreted, and while the chloride elimination falls to normal in about an hour, at the end of three hours the sulphate content of the urine is still above the normal. These results point to the sulphate as having a

direct irritant action on the renal cells. The question now is, does the kidney use up more oxygen during a sulphate diuresis than during a Ringer diuresis? Fig. 47 shows that the oxygen used increases as the amount of urine increases under the influence of sulphate, that is, sulphate diuresis entails an expenditure of energy.

The various factors dealt with above as concomitants of saline diuresis may now be dealt with in relation to sulphate diuresis.

1, 2, and 3. Increase in flow of blood, etc., do not here play a part. Bainbridge and Evans showed that in a perfused living kidney, sulphate diuresis may occur without any increase in volume.

4. The corpuscular concentration of the blood has as little effect under sulphate as under chloride injection.

5. The introduction of a colloid after a sulphate made little difference in the flow of urine, while it diminished it markedly after a chloride injection (Knowlton).

In short, filtration plays but a small part here. No doubt sulphates have a certain salt action, but this is masked by their strong secretory effect.

Function of Tubules. Next, one wants to consider what cells the sulphate stimulates. Cushny caused one kidney to secrete against a pressure of 30 mm. Hg, leaving the other kidney free. During the height of a NaCl + Na_2SO_4 diuresis he found that for equal weights of urine the obstructed kidney produced a fluid containing less chloride and more sulphate than the kidney with unobstructed ureter. The result of one experiment is appended.

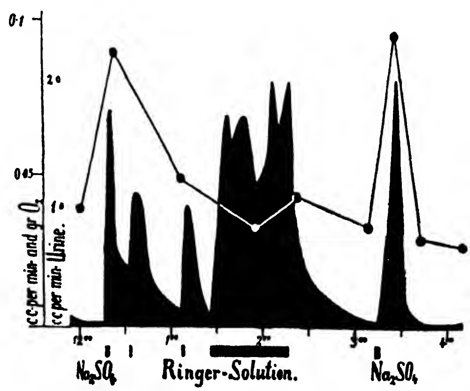


FIG. 47.—To show the relationship between the production of urine and the consumption of oxygen by the kidney under the influence of Ringer-Solution and of Sodium Sulphate. The black area indicates the amount of urine secreted, the thin line the consumption of oxygen. (Barcroft.)

	Urine Gms.	Cl. Gms.	SO_4 Gms.
(*a*) Unobstructed side . .	24	0·081	0·108
(*b*) Obstructed side . . .	8	0·014	0·067

He assumed that the filtrate from Bowman's capsule must be of identical composition in both kidneys, as each had a similar blood supply. Therefore, some change must have taken place during the passage along the tubules. In one case (obstruction) the fluid remained in contact with the lining cells for a prolonged period, while on the other side free passage was allowed. Either

sulphate must have been added to the fluid during its stay in the tubule or chloride and water absorbed from it. The two main modern theories of renal action differ on this point. The experiment is quoted at this stage to demonstrate that it is probable that sulphate stimulates the cells lining the tubule, and that their activity entails the consumption of oxygen and the expenditure of energy.

Energy Used. The consumption of oxygen, as we have seen, is an indication of energy usage, and the amount of oxygen used gives a measure of the amount of energy used. Assuming that the oxygen all goes to the oxidation of glucose, then each cubic centimetre of oxygen will cause the liberation of 0·005 Cal. from 1·5 mgrms. of glucose.

Basal Metabolism of Kidney. When the kidney is at rest, that is, when minimal amounts of urine are being formed, very little work beyond the domestic upkeep of the kidney cells is being performed and very little oxygen is consumed. A kidney in this condition, forming 0·03 c.cm. of urine per minute, used 0·4 c.cm. of oxygen corresponding to the evolution of 0·002 Cal. from the oxidation of 0·6 mgrm. of glucose.

Metabolism by Secreting Kidney. During the secretion of 2 c.cms. of urine per minute the kidney consumes about 2·4 c.cm. of oxygen. Subtract from this amount the amount of oxygen necessary for the upkeep of the cells (basal rate 0·4 c.cm. per minute) and you have the amount of oxygen used in the formation of 2 c.cms. of urine. That is, each litre of urine formed entails the consumption of about an equal volume of oxygen and 1·3 grams of glucose (approx.) with an energy utilisation of 5 Cals.

The CO_2 output varies very much even during rest and does not always increase in proportion to the oxygen absorbed. It may be that this metabolite is excreted by some other channel than the blood stream.

Work Done by Kidney. Attempts have been made to correlate the total energy exchange in the kidney with the work done, calculated from the alteration in the osmotic concentration in the various urinary constituents. The minimum work done in the formation of a litre of urine may be calculated from the Hill-Donnan formula :—

$$\text{Work} = \text{R.T.} \left[\Sigma\left(C_u \log_e \frac{C_u}{C_b} \right) + \Sigma C_b - \Sigma C_u \right]$$

where R = the gas constant, T = absolute temperature,
C_b = concentration in the blood of any constituent a, b, c, etc.
and C_u = its concentration in the urine.

This, of course, would only give the minimum work of the kidney, even if we knew the concentration and degree of dissociation of each and every urinary constituent. It may be advisable again to call the student's attention to the fact that the energy used in effecting any change is independent of the means by which that change is effected. The work done, as calculated from the Hill-Donnan formula above, is simply the minimum necessary to cause the change in the molecular concentration. It is independent of any process and commits one to no theory (see Chap. III.).

Rhorer has calculated the work done by the kidney in concentrating urea and sodium chloride, and from his figures Cushny considers that, as the concentration of urea causes the consumption of about 0·7 Cal., and similarly about 0·5 Cal. are used in concentrating sodium chloride (per litre of urine), it would not be an overestimate to state that the production of a litre of urine entails the expenditure of at least 1·2 Cals. This value, however, is but a fraction of the chemical energy used as determined by the oxygen consumption (5 Cals.). We have seen above that for each volume of urine excreted, the kidney consumes about an equal volume of oxygen. There is thus a discrepancy between the total energy absorbed and the apparent work done. In other words, the efficiency of the kidney

$$= \frac{\text{actual work done (in Cals.)}}{\text{energy used (in Cals.)}} \times 100 = \frac{1{\cdot}2}{5} \times 100 = 24 \text{ per cent.,}$$

a value closely approximating to the value found for the efficiency of muscle.

Some of the apparently "wasted" energy goes to keeping the machine warm and serves other domestic purposes. Some, again, may be used in maintaining the permanent low surface tension on the cell-lumen interface in the tubules (Macallum).

Theory of Mechanism. There are two series of facts which are very difficult to explain.

(1) Some substances occurring in blood and urine have threshold values, *i.e.*, they are not excreted till their concentration in the blood reaches a certain value, *e.g.*, water, chlorides, uric acid, glucose, etc. Others, like urea, creatinin, galactose, etc., have so low a threshold value that they may be classed with foreign solutes as non-threshold substances. Now water and chlorides are more diffusible than urea and creatinin, and yet the latter seem to be readily eliminated from the blood whenever they are present *irrespective of concentration.* In the two chapters on disperse systems we discussed the question of *free* and *bound* water. Blood plasma is a complex disperse system, and when we come to

study it (Chap. XXII.) we will see that the colloids in it have the power of binding a large amount of water and certain solutes. Serum globulin, for instance, binds a considerable amount of chloride. That is, the threshold refers not so much to a differential sill in the kidney as to the differential binding of water, organic and inorganic solutes by the hydrophilic colloids and disperse lipoid particles in the blood.

(2) The concentration of the substances in the urine differs markedly from their concentration in the plasma. Not only is this so, but similar substances, *e.g.*, potassium and sodium, undergo alterations in concentration to a different extent. The following table illustrates this point (Table XXIX.).

TABLE XXIX

Substance.	Concentration in plasma per 100 c.c.	Concentration in urine per 100 c.c.	Number of times concentrated by kidney.
Urea	25-30 mgrms.	1·8–2 grms.	60–70
Creatinin	2–3 ,,	80–90 mgrms.	40
Uric acid	2 ,,	50–60 ,,	30
Sodium	320 ,,	350 ,,	—
Potassium	20 ,,	150 ,,	7
Ammonium	1 ,,	40 ,,	40
Calcium	8 ,,	15 ,,	2
Magnesium	2·5 ,,	6 ,,	2
Chloride	370 ,,	600 ,,	2
Phosphate	9 ,,	270 ,,	30
Sulphate	3 ,,	180 ,,	60

We ought actually to have in the first column of concentrations, not the gross amount of these substances in the blood, but a much higher series of figures, viz. the concentrations of the *free* salts in *free* water. We know that the quantity of water free in the blood is remarkably small, not over 10 per cent., and probably somewhere about 5 per cent., but we have no reliable figures for the distribution of the solutes. Even if this correction were made it would account only for a few differences in concentration, and would reduce the figures in the last column quite considerably, but would not clear all our difficulties away.

The bone of contention between the two modern schools is the function of the tubule. One group holds that the tubular epithelium absorbs water and threshold salts from the fluid passing down the lumen. The other group holds that salts are excreted into the lumen by its lining epithelium. Much of the evidence produced is

of equal value to both sets of thinkers. Macallum's work, already mentioned, showing that a constant low surface tension was maintained at the cell-lumen interface rather weights the scales in favour of the second view. There is also no doubt that experiments where dyes, etc., are injected show that matter does pass, under these conditions, from tubular capillary through the tubular cells to the lumen of the convoluted tubule. On the other hand, there is nothing to hinder the reverse process from taking place if need arises. Consider the cell as middleman between blood and secretion. Any abnormality in the blood would produce an alteration in the cell, which, if it could, would pass on the change to the secretion. Let us take a concrete example. Say there is a deficit in NaCl in the tubular capillary. As a result, because the cell NaCl-*tension* must be equal to the blood NaCl-*tension*, salt will pass from the cell to the blood. Similarly, if the dialysate or filtrate in the lumen has any NaCl at all, some of it will pass into the cell to make up the deficit. The experiments of Milroy and Donnegan, already referred to, contain a series demonstrating the passing of NaCl from an aqueous solution to one containing a globulin.

Cushny's obstructed ureter experiment referred to above may be interpreted in this light. Assuming, as he does, that the fluids coming from both capsules are identical in both kidneys, then on the obstructed side there is a fluid in prolonged contact with the tubular epithelium, while on the other, this fluid passes away more or less rapidly. Take for granted, for the sake of a standard, that the freely passed urine remains unaltered, that is, it is equal to the glomerular filtrate on the obstructed side—*e.g.*, 24 grms. water, 0·08 grm. NaCl and 0·11 grms. Na_2SO_4. Sodium chloride is more diffusible than sulphate and readily penetrates cells, therefore the positive tension of NaCl in the tubule will cause some NaCl and water to enter the lining cells and so into the blood stream. The sulphate, not being so diffusible, will not so enter the cell. Thus the result would be a concentration of the urine with a decrease in chloride, *i.e.*, with sulphate steady; chloride would drop to a quarter = 0·02 and water to a half = 12 grams. But no great energy need be expended here, only sufficient to evaporate urine to half its bulk. The water and salt so secreted into the blood stream would cause a further diuresis and so on. On the other hypothesis, viz. that the sulphate is to a great extent secreted by the tubular epithelium into the lumen, this difficulty does not to the same extent arise.

In short, two factors may come into play in the secretion of urine, (*a*) the adjustment of the cell to alterations in its environ-

ment, and (*b*) a mechanical dialysis of water and crystalloids in solution through the capsule under sufficient pressure to wash the actively secreted material through the collecting tubule and on towards the pelvis of the kidney.

Other Glands of Elimination.

Of the physics of the other detoxifying glands, little or nothing is known. The largest of these is the liver, but beyond the isolation of enzymes, the physico-chemical mechanism has not been to any great extent investigated.

The intestine. Much waste matter is excreted by the large intestine and here the physico-chemical mechanism is more clearly indicated (see Chap. XXVIII.).

Skin glands—sweat excretion will be considered under regulation of temperature (Chap. XXXII.), and elimination by the lungs under Transport (Chap. XXIV.).

FURTHER READING

CUSHNY. "The Secretion of Urine." Longmans, Green & Co.

FOR RETICULO-ENDOTHELIAL SYSTEM

EVANS. "Recent Advances in Physiology." J. and A. Churchill.

CHAPTER XVII

THE CIVIL ENGINEERS OF THE BODY

CONNECTIVE TISSUE CELLS

"Which doth neatly declare how nature Geometrizeth and observeth order in all things." SIR THOMAS BROWNE.

WE have just seen how increase in the size of an organism necessitates increase in its complexity. Groups of simple naked cells held together only by a pellicle resulting from surface adsorption, would, at the best, form unwieldy organisms which would easily be distorted beyond their elastic limit and so destroyed. Some means of binding the component cells together to form a body sufficiently rigid to withstand shock and yet possessing sufficient mobility to seek its prey and avoid its enemies, is a logical outcome of growth in size and in complexity. Moreover, if the animal is to have an efficient muscular system under complete control and able to be employed under all environmental conditions, some absolutely rigid systems of portable levers and fulcrums must be presupposed.

The Vegetative Tissues are those which support, bind together, and mechanically protect the other tissues of the body. They may be divided into two groups—the epithelial tissues which protect surfaces, and the connective tissues which bind together and support the various organs of the body.

I. Epithelium.

The shape of the cells forming a protective layer or series of protective layers depends entirely on the resultant of the forces acting on them. We may take it for granted that here as well as with the single cell the agency of surface energy is obviously of first importance.

The theory underlying the phenomena associated with the manifestations of surface energy depends on the principle of Le Chatelier. Surface tension is proportional to the area of the surface of contact. It is also proportional to a coefficient which is specific for each pair of substances provided temperature is kept constant. It may be profoundly modified by the slightest alteration in one or more of the physical or chemical conditions of one or both of the phases forming the contact surface.

The form of a cell depends in great part on the magnitude of the surface forces brought to bear on it. If it is surrounded by exactly similar cells then it will tend to assume a more or less spherical form. This is exactly what one finds in the centre of a mass of soap bubbles or in the middle layers of stratified squamous epithelium. The cells are not absolutely spherical in shape, not only because the cells in mass are not absolutely similar but because the cells have to fill the space. No odd, empty spaces occur. Now, according to the principle of Le Chatelier, the surface energy will manifest itself by tending to reduce the area of contact. Mathematical proof has been given that the least possible area of contact surface is attained when the partition walls meet together in groups of three, at equal angles, *i.e.*, at angles of 120°.

The outer and inner layers differ markedly in shape from one another and from the middle layers. The outer layer is exposed to air (skin) or to the free external fluids of the body (mouth and gullet) on the one side, but is in contact with cells on all other sides. In addition, the outer surface is liable to undergo chemical changes—oxidations, etc., and physical changes—adsorption, drying, etc. These again affect tension. The result is that the outer layers are flattened and scale-like.

The form of the inmost layer of cells is governed by certain forces in addition to those acting on the more central cells. (*a*) It is obvious that the surface tension will be different at that surface where the cell is in contact with a cell differing from itself in structure and condition. These cells are in contact on either side with similar cells, but above, they press against fully grown spherical cells, while below they form interfaces with the structure on which they lie and from which they derive their nourishment. (*b*) These deeper cells are in process of division, and, therefore, one must take into account the pressures of segmentation and of growth. (*c*) The outmost layer, away from the nourishing fluids of the body, undergoes keratinisation and resists the outwards push of young cells which are thus put under stress.

II. Connective Tissues.

To appreciate the significance of the structure of the vegetative tissues, due attention must be directed to their function. These tissues are cell communities with an important but little studied industry. They are the civil engineers of the body. The structures they build are designed to withstand stresses. Before critically examining their handiwork let us study some elementary engineering problems, so that we may the better understand the phenomena of cell structures.

If we make a list of the various ways in which mechanical force can be applied in the human body, *e.g.*, by pressing, pulling, twisting, bending, etc., and examine them carefully, we find that they can be resolved into two only, viz., pressing and pulling. In attempting to bend something we apply simultaneously a compressing and a stretching force. Torsion, too, may be resolved into these two forces applied tangentially to one another.

As every substance develops within itself a force equal in intensity, but opposite in sign from the applied force, we may state that all the *stresses* developed in a material are of two kinds, *e.g.* tensile and compressive.

It is obvious that substances may be so formed that they bear up under compression, but are unable to maintain their integrity of form or of structure when pulled, or *vice versâ*. For example, a chain will withstand a great pulling force, but crumples up when compressed along its length. The stresses developed in a walking stick are parallel to its length, *i.e.* it may be pressed or pulled by forces acting axially without undue danger of fracture, but a bending force easily produces distortion. The axial forces produce stresses that lie wholly within the material and, with reasonable force, no shear occurs.

Shear stress exists between two parts of a body in contact when the two parts exert equal and opposite forces on each other laterally in a direction tangential to their surface of contact. For example, there is a shear stress on a bolt or rivet when the two plates which it holds together are pulled or pushed in opposite directions in a plane parallel to the plane of normal cross-section of the bolt.

On the other hand, force applied to a walking stick at both ends so as to bend it, very easily produces fracture. The line joining the points of application of the two forces in this case lies almost wholly outside the stick.

There are two factors to be considered when we are dealing with the ability of structures to maintain their integrity under applied force. These are (1) strength of material, and (2) nature of structure.

(1) **Strength of Material.** The ability of any material to withstand stress may be found from various elastic constants, the so-called moduli, of which there is one for each type of stress and strain.

Strain is the alteration of shape or dimensions resulting from stress, and one has a *tensile* strain resulting from a stretch, *compressive* strain from a thrust, and *shear* strain from *shear* stress. If after the stress causing a strain has been removed, the strain

disappears, then the stress has not been beyond the *elastic limit* of the material. If a stress beyond an elastic limit has been applied, part of the resulting strain remains after the removal of the stress and the material has become permanently distorted. In other words, the residual strain has become a *permanent set.* The determination of an elastic limit comes then to be the detection of the minimum permanent set. Hooke's Law (1660) states that, *within the elastic limits*, the strain produced is proportional to the stress producing it. This law holds for all kinds of stresses, but is not exactly true for all materials. The deviations, however, are few and slight. Hooke's law may be written as

$$\text{Stress intensity} = \text{strain} \times \text{constant.}$$

The constant in this equation is of the same kind as the stress intensity, and is measured in units of force per unit area. It varies (*a*) with the material stressed, and (*b*) with the type of stress developed. That is, for every material there will be a constant or modulus for tensile, for compressive and for shear stress. The modulus of elasticity might be defined as the intensity of stress which would cause unit strain.

Young's Modulus is the direct or stretch modulus. It is always denoted by the letter E. From Hooke's law—

$$\text{Stress intensity} = \text{Strain} \times E,$$

$$\text{or } E = \frac{\text{tensile stress intensity}}{\text{tensile strain}}$$

$$= \frac{\text{load on specimen} \div \text{area of cross-section}}{\text{alteration in length} \div \text{original length}}$$

Wertheim gives the moduli of the following substances in grams weight per sq. cm.

TABLE XXX.

Bone	$2304{\cdot}1 \times 10^6$
Tendon	$163{\cdot}41 \times 10^6$
Nerve	$18{\cdot}89 \times 10^6$
Muscle (resting)	$0{\cdot}95 \times 10^6$
Vein	$0{\cdot}87 \times 10^6$
Artery	$0{\cdot}052 \times 10^6$

The above values are given in order of increasing "*perfection*" and decreasing "*strength*" of elasticity. The figure last given, that of arterial walls, may be taken as substantially that of elastic fibrous tissue.

The same values are obtained for most materials for a pure compressive strain.

Shear Modulus (or modulus of rigidity, or of transverse elasticity) is the modulus expressing the relation between the intensity shear

stress and the amount of shear strain. It is denoted by the letter N (sometimes by C or G) $= \frac{\text{shear stress}}{\text{shear strain}}$.

Bulk Modulus is the ratio involved when, *e.g.*, a red blood corpuscle is immersed in a fluid pressing equally on it from all directions. That is, when three mutually perpendicular and equal direct stresses act on a body they produce a volumetric strain. The volume may be increased or decreased as the result. The bulk modulus is usually expressed as K. If the intensities of the stresses are each $= p$, then

$$\frac{p}{K} = \text{volumetric strain} = \frac{\text{change in volume}}{\text{original volume}}.$$

The volumetric strain is three times the accompanying linear strain under these conditions.

Poisson's Ratio expresses the relation between lateral and longitudinal strain. A cube of rubber, if pressed on opposite sides between the finger and thumb, bulges on the other four sides, or, conversely, if two of the parallel sides are pulled apart, the other sides become concave. That is, direct stress produces a strain in its own direction, and an opposite kind of strain in every direction perpendicular to its own. These two opposing strains resolve into diagonals, and if the body stressed ruptures it will do so along a diagonal line. This is of some importance in explaining the oblique course taken by fractures of bones due to indirect violence.

$$\text{Poisson's ratio} = \frac{i}{m} = \frac{\text{lateral strain}}{\text{longitudinal strain}}.$$

The value of m usually lies between 3 and 4.

Elasticity. All the tissues of the body are more or less elastic. This property includes (a) change of form under the action of some force and (b) the return of the body to its original form when the deforming force ceases to act.

The **elasticity** of connective tissues plays an important part in the body. (1) It is a permanent resistance to permanent distorting forces such as muscular tension and gravity. The elasticity of the intervertebral discs, and of the *ligamenta subflava*, assists in maintaining the erect posture of the body. (2) The form of tissues is preserved against the distortion due to temporary forces and intermittent forces, *e.g.*, the elasticity of the costal cartilages and of the ribs restores the chest wall to its original position when the inspiratory muscles relax. (3) Intermittent movement is transformed into a continuous movement by transmission through an elastic medium (see circulation). (4) Elasticity economises

muscular work by coming into play in the intervals between successive shocks (Marey).

Examination of Fig. 48 will reveal the fact that bodies with an elasticity like rubber act under stress in exactly the opposite way to bodies like the metals. The metals are at first resistant to the distorting force, and lengthen only slightly till the " yield point " is reached, when they lengthen rapidly with little or no increase of applied force and then suddenly rupture. Rubber, on the other hand, at first stretches easily, and then enters on a stage in which it offers greatly increased resistance to the pull and lengthens very little, as the applied force increases, till it breaks. Almost similar graphs could be prepared for compressive and shearing stresses.

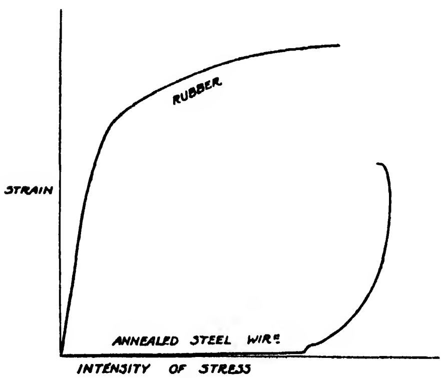


FIG. 48.—Tensile stress-strain curves for rubber and annealed steel wire (on different scales).

(2) **Nature of Structure.** A moment's thought will convince one that a quite different structure is required to meet strains of the stretching and of the thrusting varieties. (*a*) The material used in the building of struts to withstand thrust must have a high crushing limit, while that going to form ties requires high resistance to stretching. In the following table drawn up by Sir Donald MacAlister, are given the approximate values of the crushing and tensile strengths of some building materials.

TABLE XXXI

AVERAGE STRENGTH OF MATERIALS

(in kgs. per sq. mm.)

Material.	Crushing Strength.	Tensile Strength.
Steel	145	100
Wrought Iron	20	40
Cast Iron	72	12
Wood (fibrous tissue)	2	4
Bone	13–16	9–12

A glance at this table is sufficient to show that a material which may make a very good strut may make a very poor tie. This is very clear if we consider such a substance as cast iron, which may withstand a compressive force of 40 to 50 tons per square inch before it breaks, but may be pulled to bits by about one-sixth of that force. It would be quite suitable for struts, but useless for forming a sound tie-bar. On the other hand, bone is almost as well able to resist distortion by pulling as by pressing. If anything, it requires a greater

compressive force to do any damage to bone which is as it should be (Table XXXII.).

The engineer plans his structures to give the maximum strength with the minimum weight. Examination of the girders holding a roof will show that those running from wall to wall have a cross-section like the capital letter "I." This girder is a tie rod and has to withstand a stretching force. How has the engineer arrived

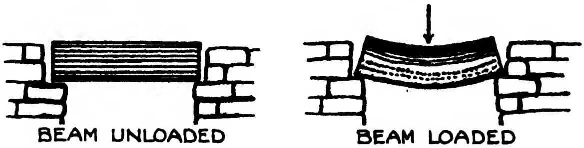


FIG. 49.—To show lines of compression (dark) and lines of tension (dotted) in a loaded rectangular beam. The clear space between the strut-lines and the tie lines indicates the neutral zone.

at this form? Fig. 49 represents a beam of square section. When such a beam is loaded midway between its supports it is slightly bent to give a concave upper surface. The upper surface is compressed while the lower is stretched. Therefore midway between the upper and lower surface lies a neutral zone or line of no stress and in its neighbourhood the material needs to have little strength. The girder maker can therefore quite safely cut away the centre of his beam, leaving only the upper and the lower surfaces, and of course some connection between them which may be almost as thin as he likes without destroying the strength. In other words, if the engineer can map out the lines of stress or directions of compression and tension in the loaded structure, all the manufacturer has to do is to see that these lines lie *in* his material; all the rest may be cut away.

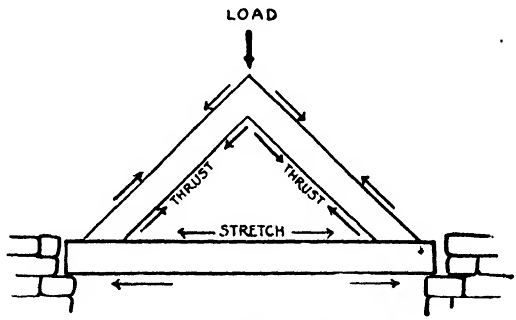


FIG. 50.—A simple triangular roof truss.

By means of the truss (Fig. 50) the simple girder becomes a tie between two struts. The horizontal member of the truss undergoes tension only, while the sloping beams are compressed. Such a structure permits of the use of two kinds of material—matter with a high tensile strength for the tie and matter able to bear up under compression for the struts.

The two principal connective tissues are fibrous tissue and

cartilage and their modifications. **Fibrous tissue** is the main binding medium of the body. It is derived from the mesoblast of the embryo. The cells of the mesoblast, which are typical spherical bodies lying close together, are gradually pushed apart by a clear transparent jelly-like exudate from the cells. They retain connection with one another by elongated processes giving the whole tissue the appearance of an attenuated sponge filled with a gel. The cells apparently secrete a colloid in a non-hydrated form which then swells up to form a gel by the imbibition of water. A model of this process may be made by adding water to a mixture of gum and oil (Part II.).

As development advances, the cells of this **mucoid tissue** become longer and more spindle-shaped (fibroblasts). The fibres are of two classes, differing from one another in chemical constitution as well as in physical properties.

(*a*) The white fibres are delicate transparent non-elastic fibres which do *not* branch arranged in bundles which do branch.

(*b*) The yellow fibres are highly refractile elastic fibres which branch and anastomose with one another. The fibres are formed by the coalescence of granules which appear in rows in mucoid tissue subjected to intermittent stretching. They have no *obvious* connection with any cell and are feebly but perfectly elastic.

The difference in their physical properties may be explained by their different chemical constitution. The former are composed mainly of a non-elastic protein **collagen** which readily takes up water to form gelatine. The latter have in place of collagen another sclero-protein **elastin**. *Though difference in chemical constitution may explain difference in physical properties, it does not make any clearer how such a difference is brought about.* If elastic and non-elastic fibres existed side by side in definite proportions one could easily mimic the formation by the separation of two colloids from a colloidal matrix. But there is no such definite proportion. Some tissues, *e.g.*, tendons, are almost entirely composed of white fibres, while elastic fibres predominate in ligaments. In short, white fibrous tissue is found where binding power alone is required, and where elasticity as well as strength is desirable, there one finds elastic fibrous tissue. *The function of the tissue governs its form.*

Just exactly how function governs form, one cannot at present say. There is no doubt that external physical forces do affect chemical actions and internal physical properties. Material under strain acts quite differently from the same matter unacted on by any force. An almost non-elastic block of rubber may be endowed with considerable extensibility by being worked with.

The optical properties of glass can be altered by submitting it to pressure. The electrical conductivity of selenium depends on the amount of light falling on it. When more is known of the laws governing matter in the colloidal state, then one may be able to give a clear answer to this problem.

In certain situations peculiar modifications of fibrous tissue are found :

(1) **Endothelium** consists of flattened cells forming a membrane. It differs from pavement epithelium by having the formed material (colloidal exudate) *between* and not *in* the cells. Such endothelial layers line all the serous cavities of the body and the lymphatics, blood vessels and heart. A structure similar to endothelium may be produced when an aqueous solution of, say, fatty acid is added to a mixture of hydrated colloids of high concentration. Under such circumstances the pressure of separation deforms the originally spherical globules to form a beaded flattened honeycomb.

(2) **Fat Cells.** The experiments detailed in Chap. IX. throw light upon the appearance of fat in the cells. There is scarcely a tissue or fluid in the body that does not contain fat in amounts in excess of the quantities that can be dispersed in colloid-free water. Finely divided fat in cell protoplasm is comparable to an emulsion. It depends for its permanence on the same factors as maintain fat in a finely divided form in an aqueous dispersant, *i.e.*, mainly on the presence, in the tissues, of hydrophilic colloids. While the fat *in the cells* is not ordinarily visible or even demonstrable by microchemical methods, when an excessive amount of fat is present it may be seen in the network of areolar fibrous tissue, especially round the smaller blood vessels. Little droplets of oil at first appear and these become larger, run together and coalesce, forming a single large globule, distending the cell and pushing to the sides the protoplasm as a sort of capsule. Reference to the chapter on emulsions will show that when the *colloid* in an oil-in-colloid emulsion decreases in hydrophilic power beyond a certain amount, the nature of the emulsion is changed to colloid-in-oil. This latter emulsion differs from the former not only in the visibility of the fat, but in this respect that the fat may be stained (black) by osmic acid or (orange) by sudan III. (page 105.)

In starvation the fat gradually disappears from the cell leaving the hydrated colloid, which also in time disappears and the cell resumes its shape.

Apart from acting as a storehouse of energy, fatty tissue has important mechanical functions. As we shall see later, the layer of subcutaneous fat serves as an extra garment protecting the wearer from the too rapid loss of heat (Chap. XXXII.). Then too,

fatty fibrous tissue has a considerable amount of resilience, acting as a buffer protecting organs from external violence.

(3) **Pigment Cells.** Fibrous tissue cells (and other cells) in certain parts of the body (*e.g.*, eye) may contain a pigment—**melanin.** How this pigment is formed and what exactly are its functions remain matters of conjecture. Chemically, melanin is closely related with the **melanoidins**—dark pigments resulting from digestion of proteins with hot mineral acids. They serve (*a*) as light filters—preventing the passage of light through the pigment cell. That is, the pigment absorbs energy. These pigmented areas are nearly always found in places exposed to light, and one may suppose that the incidence of strong light on fibrous tissue may cause the formation of melanin from the cell protein. Inorganic examples of the formation of light-absorbing chemical compounds by the absorption of light will occur to the student (cf. Silver Salts). (*b*) Their function is not only to protect the underlying tissue from the harmful action of radiant energy, but in many cases the pigment cells act as a transmitting station—receiving the light stimulus and transmitting it to the effectors. This **dermatoptic function** has been studied and described by R. Dubois. Light falling on the pigment cells of the epithelium of the siphon of *Pholas*, a mollusc, causes a reflex retraction of the siphon. Observation under the microscope has shown that the pigment cells in the skin of the frog contract when light falls on them. The pigments of the eye and their action in transmitting light stimuli will be dealt with in another chapter.

Two modifications of fibrous tissue warrant separate treatment, *i.e.* **cartilage and bone.** In lower animals and during the fœtal life of higher animals (as well as in certain situations in adult life) rigidity is given to the body by cartilage. The function of cartilage cells will be dealt with later under bone and lubrication. Here it is sufficient to note that the peculiarity of this tissue is the secretion of a homogeneous translucent gel which is tough and elastic. This *chondro-mucoid* material is a mixture of at least three colloids: ordinary protein, collagen and chondroitin. On decomposition this latter substance yields substances of a carbohydrate nature, glucosamine and glycuronic acid (cf. emulsions).

(4) The great supporting tissue of the body is calcified fibrous tissue or **bone.**

(i.) **Development.** Bone is formed by a deposition of calcium salts in white fibrous tissue. Some bones which are more or less flat, *e.g.*, vault of the skull and the scapula, are formed directly in fibrous tissue. This is the so-called *intra-membranous bone formation.* The long bones are *preformed in cartilage* into which

processes of fibrous tissue find their way and they in turn undergo calcification. *All bone is developed from fibrous tissue.* The cartilage merely plays the part of scaffolding and is all replaced by fibrous tissue before ossification takes place.

TABLE XXXII

RELATIVE STRENGTH OF THE LONG BONES

(MAN AGED 31)

Bone.	Crushing Strength (in kg. per sq. mm.).		Bending Strength (in kg.).		Shearing Strength.		Place of Rupture.	Torsion * (in kg.).
	1st appearance of breakdown.	Complete crushing.	Max.	Min.	Max.	Min.		
Humerus	8·5	—	300	120	505	250	At ends . .	40
Radius .	5·3	—	140	55	334	105	In middle .	12
Ulna .	5·5	—	140	70	235	90	Anywhere .	8
Femur .	13	29	475	230	810	400	At the neck .	89
Tibia .	6·0	42	500	135	1060	450	At the lower end	48
Fibula .	3	—	55	21	61	20	In the middle .	6

* Torsion applied to the extremity of the bone with a leverage of 16 cm. produced a spiral fracture with the forces given above. (Amar.)

Practically nothing is known of the physical chemistry of bone formation. Microscopic investigation suggests to our mind a process similar to the formation of a honeycomb. The cells of fibrous tissue detailed to build bone, *i.e.*, osteoblasts, secrete material containing a fair proportion of the phosphate and carbonate of calcium. It is known that the presence of a small quantity of a colloidal complex alters the solubility of inorganic matter. For example, calcium phosphate is more soluble in an albuminous hydrogel than in water. This effect is even more marked with calcium carbonate. If we presume the presence of the salts of lime in the fibrous tissue cells, then, by the principle of Willard Gibbs, they will be found in greatest concentration where the surface tension is lowest, that is at the cell borders. Another factor may be brought into play, viz., alterations in the colloidal matrix. Albumin is broken down in the body to proteoses and peptones. Now, experiment has shown that calcium salts dispersed in an albuminous hydrogel are thrown out of solution when protesoses and peptones appear in the gel. Further, calcium phosphate is much more insoluble in proteose-peptone solution than the carbonate, which is only slightly affected by the change. It is significant that bone ash contains about 84 per cent. of the former and only 7·6 per cent. of the latter salt.

One cannot say why cells in certain situations should have this property of ossification. How far stresses and strains affect the process is unknown. This we do know, however, *that the internal structure of the bones undergoes alterations to suit alterations in the application of external forces.*

(ii.) **Internal structure.** In the earlier part of this chapter mention was made of *lines of stress,* and it was there stated that as long as sufficient strong material was present to include the course of these lines it was an obvious economy to cut away as much as possible of the matter in which there were no stress lines. If these lines lie wholly in the structural material, then the danger of rupture under shearing stress is eliminated. *A shearing stress* is a force which tends to cause one part of a structure to slide over another part. For example, a pile of coins compressed by a force acting at right angles to the face of the coins effectively resists the compression. If, however, the force were to act obliquely to the face of the topmost coin, it would immediately cause the pile to slip asunder. *In other words, a shearing stress is ineffective along the lines of maximum compression.* The same can be demonstrated for lines of maximum tension. For all other lines, shearing stress has a definite value which is obviously at maximum at 45°, *i.e.*, half-way between the lines of tension and compression. Professor Culmann, an engineer from Zurich, happened to see some drawings by Professor H. Meyer of the cancellous tissue of the femur and at once noticed how the trabeculæ of the bone coincided with the lines of stress. He gave his class of engineering students an outline of the femur and told them where the stresses fell. He asked them to draw the internal structure which would be necessary to meet these stresses. Fig. 51 shows the result. Alongside this figure is given a diagram of the Fairbairn crane—one of the best weight-lifting mechanisms known. The similarity between the natural and the artificial structures is obvious. It will be noticed that the lines of the trabeculæ of the femur run in two systems of curves. One system runs along the outer convex side of the shaft, curves downwards as it opens out with concavities downwards. The other system starts from the inner side of the shaft and rises spreading outwards with the concavities upwards. These systems correspond to the two kinds of lines of stress present, *e.g.*, tension and compression. The convex or outer side has to resist tension, while the inner convex side, overhung by the loaded head, is the compression member. The head of the femur is a little more complicated than Fairbairn's crane, in that the load is applied on two points, *i.e.*, on the head of the bone and on the great trochanter. This entails a division in

the distribution of the stress lines corresponding to the incidence of the loads. In the compact tissue of the shaft the tension and compression lines run parallel. The lines of stress are closest together at the point of greatest strain, *i.e.*, in midshaft. This place has to be thickened to prevent the bone from snapping (a walking stick pressed vertically against the floor breaks halfway up). The central portion of the shaft has to bear no strain, and therefore is hollow. It may be considered as a large mesh between the tension and compression lines. In the cancellous

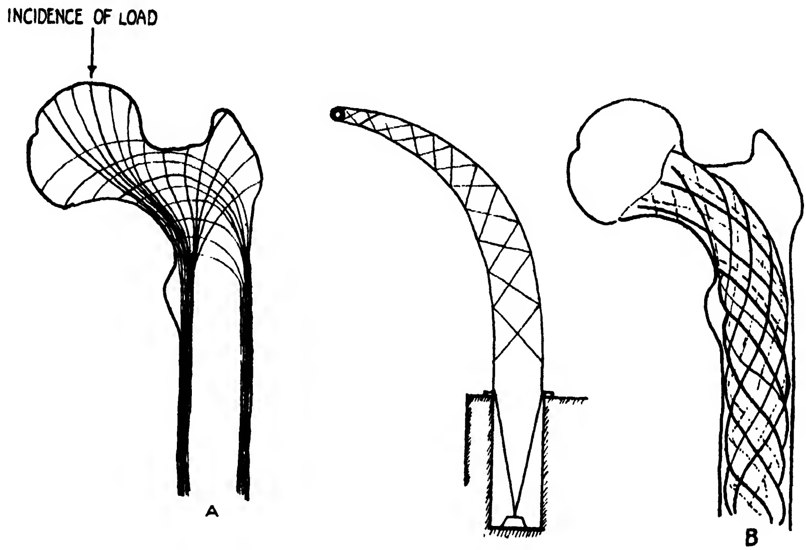


FIG. 51.—To show the stress lines in the head of the Femur, *A*, in section, and *B*, on the surface. The central diagram gives an idea of the location of the lines of stress in the head of a crane. (After Culmann, Meyer and Dixon.)

tissue the tension lines cross the compression lines at right angles.

The same phenomenon may be seen in any bone which undergoes tension and compression. It is very noticeable in the human foot, especially in the heel bone (calcaneus). It is roughly triangular, having three bearing surfaces. The upper surface is compressed by the weight of the body applied from the ankle bone. Therefore, compression lines start from it and run downwards. The lower surface rests on the ground, *i.e.*, has to bear an upward thrust, and so compression lines run upwards from this surface to meet the compression lines from the upper surface. The third or anterior surface is in contact with the bones of the arch of the foot and transmits the ankle pressure forwards to them. This gives rise to a second system of compression lines running obliquely forwards. These two systems correspond to the two beams in

Fig. 50. Now the application of a load at the apex would cause the beams to diverge at their lower ends if they were not tied together by the girder. So tension lines must exist to prevent the fracture of the bone between the two systems of compression lines. These tension lines will be seen in Fig. 52 forming curves with their concavities upwards and orthogonal to the compression lines. They are continued forward by the fibres of the plantar ligaments which act as the tie-bar of the arch. It will be noticed that these ties in the calcaneus are closer together at the arch of the bone between the two struts, *i.e.* at the point where

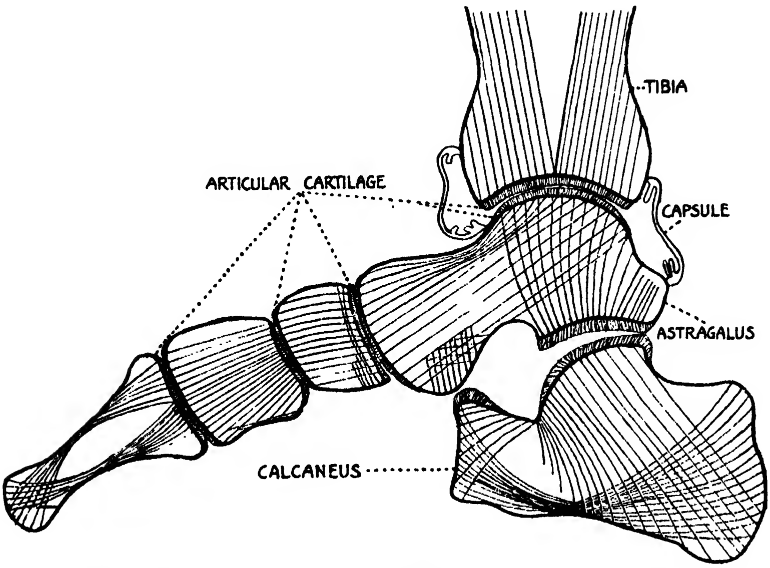


FIG. 52.—Diagram showing some of the stress lines in the arch of the foot. (After Hermann Meyer.)
(The diagram is not strictly a section, and the stress lines are not all in one plane.)

fracture is most likely to take place. Just above this point no stress lines can be seen, *i.e.* there is a neutral region. Examination of the bone makes clear the fact that in this neutral zone trabeculæ are almost entirely absent. Where there are no stress lines it would be a waste of material to build struts or ties. As Sir Donald MacAlister puts it, "*any mass of bone put there would not 'row its weight,' and it has been 'turned out'.*"

This internal structure is altered to meet alterations in the incidence of stress. For example, during the first twenty years of life when the body is growing and the bone lengthening, constant alterations in internal and external structure have to be made. The unnecessary parts are decalcified and the fibrous tissue undergoes alteration. During this process some of the fibrous tissue

cells become enlarged and multinucleated. Histologists call these cells *osteoclasts*. An adjustment to meet altered conditions may be seen when a bone is broken and allowed to set badly, so that its parts lie somewhat out of their former positions. Tension and compression lines do not now coincide with the trabecular structure. It has been shown by Wolff and others that in a few weeks, not only has an alteration taken place at the seat of fracture, but *the entire trabecular system, right to the ends of the bone, has undergone remodelling to suit the new incidence of forces.* More recent work on bone grafting has amply demonstrated the astonishing rapidity with which reconstruction of the trabecular meshwork takes place. One must remember that in spite of its rigidity, bone is plastic. Physical chemists have proved that when an inorganic constituent separates as a definite phase from a colloidal matrix, the new phase is at first liquid. We may, therefore, infer that the new trabeculæ are more or less liquid when formed. The action of force upon them will tend to set them along the lines of that force, *e.g.*, straws set along the direction of the wind. They are practically "carded" into position, where being in equilibrium they will tend to "solidify" in mass.

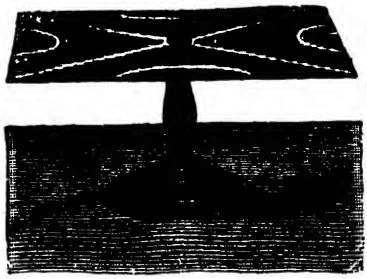


FIG. 53.—Chladni's experiment.

One may easily see this process of "carding" in action in the siliceous sponges. These sponges not only orient themselves in water so as to reduce their resistance to the steady dominant flow of their environment, but their siliceous skeleton which supports the softer tissues has an internal structure like bone with the silica laid down on stress lines so arranged as to take the load. Altering the direction of the stream of water and thus setting up stresses different from the previous ones, causes the laying down of a new skeleton with an absorption of the old one. Chladni's experiments (Fig. 53) demonstrate that fine grains are deposited on a horizontal vibrating plate in certain patterns or figures, depending on the nature of the vibration produced in the plate. That is, the plate does not vibrate in all its parts at the same rate. Some points or nodes are practically stationary, and it is at these nodes that most grains are found. Dendy and Nicholson observed that siliceous particles were first laid down on the nodal points of the sponge. The basis, then, of each line of stress outlined in silica is a series of points which remain stationary when the sponge

vibrates on the impact of a stream of water. There is quite a body of evidence that a similar vibration-theory may hold good as well for the work of the osteoblasts as for that of their ancestral relatives the scleroblasts. Compare, for example, the interlacing spiral lines in which calcium is deposited (Fig. 51, B) in the femur with the similar figure obtained when sand is scattered on the surface of a rectangular bar clamped horizontally at one end and caused to vibrate. The nodal lines are really lines separating two parts which are vibrating in opposite phases and so correspond to the stress lines. If now we dust the plate with two powders, one heavy, say, blue sand, the other light like lycopodium, we will have a pattern in blue on the nodal lines and a pattern in white at the parts of maximum vibration. This deposition of the light powder is due to the formation of small vortices in the fluid (gas or liquid) near the vibrating body sweeping the powder to the centre of the vortex (Faraday). Bone is subject to longitudinal, transverse and to torsional vibrations, and has in it all the factors to produce vibration figures.

Fractures. There are several ways by which a piece of blackboard " chalk " can be broken. Let us consider three of them. (*a*) If we hold it by both ends and apply force directly at right angles to the long axis of the chalk, it will snap transversely at the point of contact of the distorting force. (*b*) An oblique fracture results from smacking the chalk vertically on the table. The force of compression applied along the long axis leads to an extension at right angles to this (cf. rubber cube, p. 207). The fracture is only indirectly due to the impact on the table, but is directly due to the bulging being beyond the elastic limit of the material. (*c*) A twisting force applied to the chalk produces a spiral fracture. These types of fracture can all be produced in bone. (*a*) Fractures caused by direct violence are transverse and are located at the point of incidence of the force. (*b*) Oblique fractures are a sign of indirect violence, *e.g.* fracture of the clavicle from a fall on the outstretched arm. (*c*) Spiral fractures are produced when the body is twisted with the limb fixed.

(5) **Lubricating Cells.** Certain cartilage cells have a peculiar function, that of acting as a lubricant between rubbing surfaces. One of the most worrying problems of the engineer is to prevent " heating up " of moving surfaces. This he attempts to do by interposing a fine uniform film of oil between surfaces where friction is apt to take place. The particles of the oil film act as microscopic ball bearings over which the moving surfaces slide with the minimum of friction. The motor cyclist knows how essential it is to have the right amount of the right grade of oil in the right place.

In spite of all precautions, however, " seizing " does take place. The film of oil is rubbed away just at the point where it is most required. Only one machine has, as yet, been designed which has a perfect lubricating system, and that is the animal body. In the body there are many rubbing surfaces. At joints, bone works against bone : tendons run like Bowden wires in sheaths, and yet the healthy animal body moves noiselessly and without " heating up " or " seizing " at any speed.

(*a*) **Joints.** There are, counting great and small, 230 joints in the human body varying in degrees of magnitude and importance. The ends of the two opposed bones in a joint are coated with a thin layer of cartilage. This cartilage, in the adult, is what is left of the scaffolding of bone. As we have seen, it is elastic and acts as a resilient buffer. The surface is always covered, in health, with a film of synovial fluid.

This synovia is kept in place by being enclosed with the joint in a flaccid membrane or joint capsule (Fig. 52). The synovial fluid results from the destruction of the cartilage cells on the rubbing surface of the joint. In this way the supply of lubricant is absolutely automatic. The more the joint surfaces move on each other, the greater is the destruction of the cartilage cells and the more plentiful is the supply of synovia.

Two other points require our attention, (i.) How is the supply of synovia kept up and (ii.) what happens to the waste fluid. (i.) The cartilage is constantly, like epithelium, growing. The young cells take their origin in the layer next to the bone and push their way up towards the outer surface of the articular cartilage. Every cell destroyed to form synovia has its place taken by a cell from the layer below and so on. Growth and destruction exactly balance one another. (ii.) The waste synovia is drained into the blood stream through villous processes which project from the synovial membrane into the cavities of the joints.

(*b*) **Tendon sheaths.** Muscles are attached to bone by sinews or tendons, and these cordy structures work in sheaths. The inner surface of the protecting sheath as well as the outer surface of the tendon is endowed with a lubricating substance similar to that of the joints.

There is one outstanding point of interest about the lubrication system of the body and that is its nourishment. As far as is known all other cell communities draw the material they require for maintenance and growth from the blood stream. As we shall see in the sequel the red blood corpuscle performs the duty of oxygen carrier. No red corpuscles enter articular cartilage—the gristle in joints is pearly white. One can only suppose that the

plasma which reaches the cells from the rich vascular network on the surface of the underlying bone, carries dissolved in it sufficient oxygen to meet the needs of these lubricant-formers, as it carries sufficient protein, carbohydrate, etc., for their use.

The formation of cartilage and of synovia and the relation of these two substances to bone and to fibrous tissue is a rich field for investigation. Certain colloidal phenomena will occur to the student as suggestive of an explanation, but absolutely no definite physico-chemical facts can be brought forward as acceptable evidence.

Further Reading

Thompson. "Growth and Form." Cambridge University Press.
Keith. "Engines of the Human Body." Williams and Norgate.

CHAPTER XVIII

THE INTELLIGENCE SERVICE

NERVE CELLS

"The messengers that preserved a communication between the soul and the outward members." BERKELEY.

It is obvious that in an organised conglomeration of cell bodies like the animal body some means of rapid communication must exist between one organ and another. Without it, rapid co-ordinated movement by the body as a whole would be impossible. This work is accomplished by the nervous system. Two entirely different systems of rapid communication exist in the body. One runs to and from the body wall and has to do with the relation of the body to its environment. It belongs to the army of defence and defiance. The other system of rapid communication conveys messages to and from the industrial communities.

Embryologically, communication between an inland cell and the outer world is effected, in the first instance, by an ingrowth of the external epithelial covering. That is, messages are passed on to the inmost cell by a file of cells, detailed for this service. These cells are, to begin with, all structurally and functionally alike (neuroblasts). Later, some few of them send out long processes towards the surface and towards the organ. These processes end in branching twig-like structures called dendrites (Gr., a tree), through which they seem to be able to pass on stimuli to one another. The name **synapse** (Gr., a junction), is given to the juxtaposition of the dendrites of a nerve cell or cyton, with the terminal processes of the axon of another cyton. The cell with its processes is called a **neuron**. (See Fig. 54.)

The second system of nerves, that of the viscera, is formed of neuroblasts which have migrated towards the organ from the neural canal. They all pass through at least one **ganglion** or a **plexus** which acts like a local headquarters or exchange.

Some nerves have a sheath or coat composed of unsaturated fatty acids and lecithin (and allied lipoids). This medullary sheath is formed from separate cells, but it must retain some connection with the nerve cell, as it dies and disintegrates when dissociated from it.

This sheath is present on the nerve fibres which are concerned with rapid adjustments to alterations in environment. Peripheral non-medullated fibres generally belong to the second system of nerves and have a slower reaction rate as befits the slower adjustments of the structures they innervate.

1. **Structure.** A nerve is really a compound structure composed of parallel bundles of nerve fibres (funiculi), each of which is enclosed in lamellar connective tissue (perineurium). The nerve fibres in a funiculus are supported by a delicate fibrous investment —the endoneurium. The whole collection of funiculi—the nerve—is united together and to neighbouring tissues by epineurium.

Each fibre is an anatomical and physiological unit, and runs an uninterrupted course between central cell (brain, spinal medulla, sympathetic ganglion, etc.) and peripheral end organ (in muscle, gland, sense organ, etc.). Each consists of a long thread of protoplasm (axon) drawn out from and continuous with the cytoplasm of the nerve cell.

The neuron (cell and fibres), like any other cell, is a colloidal fluid mass. (i.) This may be demonstrated by examination of the living nerve by means of the ultra-microscope, when particles in Brownian movement will readily be seen. Some of these particles at times clump together to form large aggregates which again dissociate. (ii.) Carlson has shown that nerves may be stretched without altering their efficiency, judged by rate of conduction of an impulse. (iii.) Macallum states that alterations in surface tension can be detected especially in the growing nerve. (iv.) It has been urged by Gothlin that, as a nerve is doubly refracting to a slight extent just like muscle it must have a similar composition. These facts all go to prove that nerve is of a liquid nature.

2. **Its function** is to conduct. One cannot lay too much stress on the fact that it does not conduct an impulse originating outside, as a telephone wire conducts current from a battery. *The battery is an integral part of the neuron* (cf. Irritability of Cell, Chap. XII.).

3. **The nature of the stimulus** seems immaterial. Mechanical, electrical or chemical stimuli all cause the nerve to propagate the same kind of impulse. Furthermore, the excitatory result of the propagated impulse depends not on the nature of the " trigger " stimulus but on the nature of the mechanism to which the nerve goes. That is, stimulation of the sciatic nerve by electrical, mechanical, thermal or chemical means causes contraction of the gastrocnemius muscle; stimulation of the vagus nerve by any means slows the heart, stimulation of the chorda tympani causes the salivary glands to secrete, no matter how the stimulation is effected. These are all outgoing nerves. The predominating fibres in them carry impulses to the periphery to produce action of

some sort. A similar investigation may be held concerning those nerve fibres which carry impulses from the periphery. Stimulation of the optic nerve gives rise to the sensation of light, of the cochlear nerve to sound, and so on. On these facts about sensory stimulation is based *Müller's "Doctrine of Specific Nervous Energy,"* which reads : " The specific sensations of each sensory nerve can be evoked by different internal and external stimuli." " Sensation is not the transmission to consciousness of a quality or state of an external body, but of the quality or state of a sensory nerve as produced by an extrinsic cause, and these qualities differ in the different sensory nerves." They differ not because of any inherent quality in the nerve, but because of the nature of the central body to which they go. In the same way, as far as modern work goes it shows that the concomitants of all nervous impulses are absolutely similar. The sole difference between motor neurons and

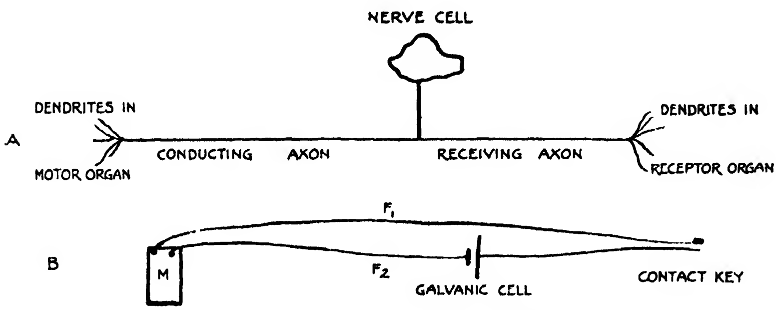


FIG. 54.—A. Diagram of a unit of the nervous system compared with B.
B. Electrical Model to illustrate Müller's Law and the " All or Nothing " hypothesis as explained in the text.

sensory neurons is in the nature of the organ to which the nervous disturbance is propagated.

No difficulty should be experienced in grasping this idea, especially if an electrical model be kept in mind.

Consider an electrical circuit such as shown in Fig. 54B, where a galvanic cell or other electrical unit is connected by wires F_1 and F_2 to M, an electric machine. A key closes the circuit. (*a*) It does not matter how the key is closed, the current passing along F will be the same, and (*b*) the manifestation of the current will depend on the nature of M. If M is a telephone receiver, the closing of the key will cause a sound to be heard, if M is an incandescent globe, light will be seen, if M is a motor, motion will result, and so on. The electrical energy of the electric generator can thus be converted into any form of energy by an appropriate M. Further, the magnitude of the force applied to the key makes no difference to the magnitude of the resulting manifestation at M.

That depends on the energy set free by the cell and on the resistance of the circuit.

Of course the receptor must be modified to suit different kinds of stimuli. A telegraph key or a bell push is a convenient kind of mechanism for closing a circuit mechanically, but it would not answer as well for electrical, thermal, sound or light vibrations. Special means for closing the circuit have to be devised to suit different kinds of stimuli. For example, sound waves may be caused to close an electrical circuit by microphone, *e.g.*, telephone transmitter (see following chapter).

The neuron may be likened to this electrical model (Fig. 54A). The nerve cell is similar to the galvanic unit, *F* is the axon or

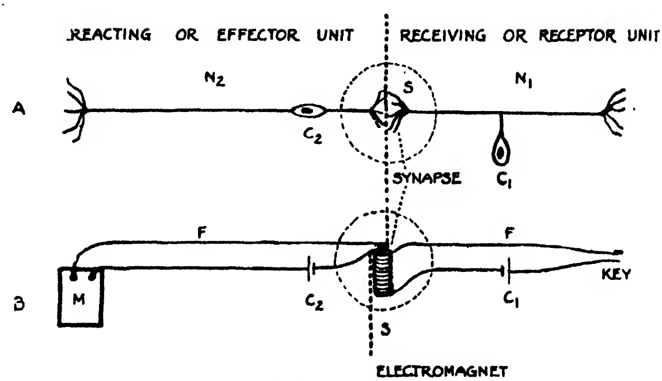


FIG. 55.—A. Diagram showing a *receiving* (N_1) and a *reacting* Neuron (N_2), each with dendrites at its extremities, and their connection to one another through a Synapsis (S). B. Electrical Model to illustrate the functional continuity of two neurons. See text.

nerve fibre, the key the receptor mechanism and *M* the effector mechanism.

A second circuit of the nature of a telephone relay could be added to the first, *S* being an electro-magnet which closes the second circuit when the key of the first is depressed (Fig. 55). *S* may be termed the synapse joining an effector and receptor neuron. The student will notice that there is no material continuity between the two neurons and that no energy passes from one to the other.

4. "All or nothing." It is obvious in the electrical model that connection is either made or not made. The energy available from C_1 is a fixed quantity independent of the energy used to close the circuit, and similarly the energy in the system of which C_2 is the cell is independent of the energy used in the electro-magnet *S*.

It is true also for the nervous system that the *maximum* motor effect is produced, if any effect is produced at all. It is a case of "all or nothing." The existence of the "all or nothing" effect

is due to differences in the structure and diameter of fibres. The former has been referred to on p. 222; as regards the latter, we may say that velocity is a linear function of the diameter of similar fibres. The rate of transmission also alters with the temperature level, as does the duration of the local electrical disturbance.

8. Cause of the electrical change. It is generally admitted that the action potential difference is the *sign of a local ionic change* in nature similar to that occurring in other protoplasmic units in action. In the first place, let us consider why the electrical change is *local*. That is, the nerve fibre is not to be looked upon as a simple electrical conductor, but rather as a chain of membrane-bounded chambers containing ionised protein, ionised salts, etc., and developing a local Donnan membrane-potential (cf. muscle fibrillæ). When one of these chambers is "excited" there is a heaping up of ions on the membrane separating it from the next chamber in order of progression, and this in some ways leads to an alteration in permeability—an injury of the membrane, and the disturbance passes to the next chamber, and so on. The evidence for the local nature of the change is primarily twofold.

(*a*) *Resistance.* The resistance of the nerve fibre to the conduction of an electric current is about 10^9 ohms per centimetre, and varies with the cross-section of the fibre. According to Ohm's law, the current flowing in a conductor varies directly as the potential difference (E), and inversely as resistance (R) of the conductor, *i.e.* $C = \frac{E}{R}$. If, for example, the value of 1 volt be given to E, then the current flowing in a fibre 10 mm. long would be 10^{-9} amperes. We have no means at present of computing the E.M.F. developed in nerve, but it would need to be well beyond physiological limits to produce a current flow in, say, the sensory nerve fibre bringing the sensation of tickle from the sole of the foot.

(*b*) *Decrement of the nervous impulse.* If a length of nerve is cooled, not only does the velocity of the propagation of the impulse suffer diminution, but there seems to be a diminution in the intensity of the impulse as well. If the degree of cold is sufficient, or if the length cooled is extensive, the impulse may be stopped entirely. If, however, any of the impulse is propagated through the cooled region into a normal piece of nerve it seems to recover its full intensity and velocity. Lucas compares this phenomenon to the transmission of fire along a fuse of gunpowder. If a section of the fuse is slightly damp, the rate of burning as well as the heat evolved will be decreased, but will recover as soon as combustion starts on a dry section. Narcotisation of a nerve by ether, alcohol, cocaine or other drug has a similar effect

to cooling. The firing of a train of gunpowder is a series of local acts. Moreover, an increased resistance in a straight electrical circuit would produce a decrement of current which would not recover its pre-resistance value no matter how good a conductor it passed into.

9. **Refractory period.** The passage of a nervous impulse produces some change in the physico-chemical state of the nerve, so that it is followed by a state during which its function is depressed. A certain time must elapse between each nervous impulse. This spare time is called the refractory period, during which a stimulus will not receive normal treatment. The length of the period varies inversely as the temperature. The refractory period may be divided into three stages: (*a*) The ***absolutely*** refractory period when no stimulus, however strong, is effective. (*b*) During the *relative* refractory period the nerve is recovering and will respond to stimuli of supernormal strength. (*c*) The *supernormal* stage follows during which subnormal stimuli are effective. Two factors at least come into play to cause the refractory period, viz. alterations in excitability and alterations in conductivity. These two factors go hand-in-hand, *i.e.* the nerve is non-irritable and offers a resistance to the passage of the impulse sufficient to swamp it during the absolute refractory period; during the relative refractory period the nerve steadily recovers its irritability and its conducting power; while the last period is one of supernormal irritability and conductivity. Take a very simple analogy. In a game common to Boy Scouts, and, I understand, borrowed from the Navy, a row of players are so arranged in unstable equilibrium that on the word or sign of command (stimulus) number one falls towards number two, and so on, till all the players are horizontal. The disturbance has been propagated from one end of the line to the other. Note in the first instance that the stimulus has not supplied any of the energy. Secondly, that the disturbance alone has been propagated, and that each unit of the team has supplied its own energy (+ gravity). Thirdly, before a second impulse can be propagated *all* the players must be restored to the vertical. Until this is done, no amount of stimulation is of any use. This is the absolutely refractory period. We cannot push the analogy further.

10. **Summation.** If a second stimulus be applied to the nerve during the third phase of the refractory period, it will give rise to an impulse which will meet with less resistance in its passage along the nerve. Now, if the first impulse be subminimal, *i.e.*, insufficient to cause a manifestation of energy in the motor mechanism which the nerve supplies, then the second impulse if it be propagated along

a nerve during the supernormal period may cause the motor end-organ to act. Such a phenomenon is called summation.

11. **Fatigue.** Nerve fibres can apparently act as conductors of the nervous impulses for very long periods without showing any signs of fatigue. It is generally said that nerves cannot be fatigued. While this is true of the conducting power of the fibre it is not applicable to the neuron as a whole. (1) The nerve cell loses something in the process. Granules which are apparent while the cell is at rest diminish slowly during activity. Then (2) changes take place at the synapses, the junction between neuron and neuron, and also at the "end plate" or junction between nerve fibre and organ. These potential junctions lose their power to cause the impulse of one neuron to act as stimulus to the next neuron or to the end-organ. They become fatigued.

12. **Metabolism.** This leads one to infer that the energy exchanges during the conduction of impulses are small. There is no doubt of the need for oxygen for the metabolic changes of the nerve cell, but the extra amount necessitated by the passage of a nervous impulse has not been estimated. Ingenious methods have been devised by Waller, by Tashiro, and by Hill for the measurement of the CO_2 evolved during activity. Hill has found that the heat and CO_2 liberated in nerve activity represent an appreciable amount of metabolic change.

13. **Temperature coefficient of the nervous impulse.** When a length of nerve is cooled its power to conduct an impulse is decreased; that is, nerve-conduction has a positive temperature coefficient. It was pointed out by Van't Hoff that the velocity of chemical reactions is increased twofold or more for each ten degrees in temperature (Centigrade scale), *i.e.*, the temperature coefficient for chemical reactions is greater than 2. On the other hand, the temperature coefficient for physical processes is less than 2. The temperature coefficient, *i.e.* ratio of velocity of propagation of nervous impulse at $(T + 10)°$ to its rate at $T° = \dfrac{v \text{ at } T + 10°}{v \text{ at } T°}$, has been estimated by Lucas as approximately 1·8. This value has been proved to be right by later workers. Therefore, physical factors, as well as chemical reactions, are involved in the propagation of a nervous impulse.

14. **Polarisation.** (*a*) *Negative polarisation.* A disturbing artefact is produced when a medullated nerve is stimulated and leads are taken to a galvanometer from two points on its length. In Fig. 56 we have a diagram of such a circuit. The upper circuit is the polarising one, *i.e.* at the anode, positive charges develop in the sheath (due supposedly to the dissociation of electrolytes by the

passage of the current through the sheath). Similarly, below the cathode there is a collection of electrons. If now the current is switched off and the lower circuit (through a galvanometer) switched on, it will be found that a potential difference has developed, causing a current to flow through the galvanometer in the opposite sense to the polarisation current. The previous anode has become the cathode, *i.e.* the anode has been positively polarised and the cathode negatively polarised.

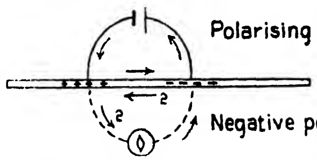


FIG. 56.—Diagram to show direction of the negative polarisation current in a medullated nerve.

Polarisation model. The theory that the phenomenon is due to a heaping up of opposite charges in the sheath is supported by a simple inorganic model (Fig. 57). If for axon and sheath we substitute a zinc wire and some cotton wool soaked in saline, we would find that the cotton wool would collect positive charges under the anode, negative charges under the cathode, and show a negative polarisation in the same way as medullated nerve. The substitution of zinc sulphate for the sodium chloride in the cotton " sheath " prevents polarisation, indicating the probability that the phenomenon is due (1) to the sheath, and (2) to some ionisable substance therein.

When an electric field is developed in water, each water molecule in the field becomes polarised, *i.e.*, oriented so that the positive ends (H) all point in one direction, and, of course, the negative ends (OH) in the opposite direction. The result of this arrangement is the formation of a large number

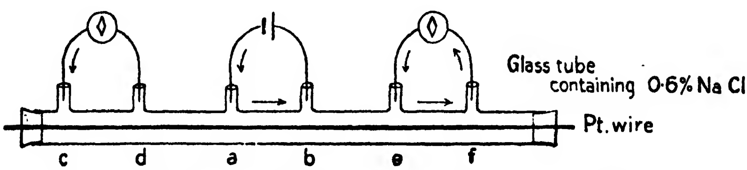


FIG. 57.—Apparatus for imitating the polarisation phenomena in medullated nerve.

of tiny condensers in series stretching from one electrode to the other, thus giving water a high value as a dielectric (*q.v.*). The polarity of the water molecules, in other words, the presence of efficient condensers, may account for the power water undoubtedly has of causing the dissociation of electrolytes into ions (*q.v.*). If we measure the charge on a condenser formed by immersing two metal plates in pure, air-free water, and get a value, we can then alter the value by dissolving various salts in the water. The addition of salts, ionised proteins, etc., increases the potential of the condenser. A charged condenser can, of course, give up its charge (cf. Leyden jar). This is apparently what happens when a current is passed through the medullated sheath of a nerve. The discharge of the condensers gives rise to the negative polarisation current.

Electrotonic Currents. That negative polarisation occurs while the polarisation current is running may be shown by an experiment as indicated in Fig. 58. The centre circuit is supplying the polarising current, x being the anode, and y the cathode. The two lower circuits are merely leads to galvanometers, G_1 and G_2.

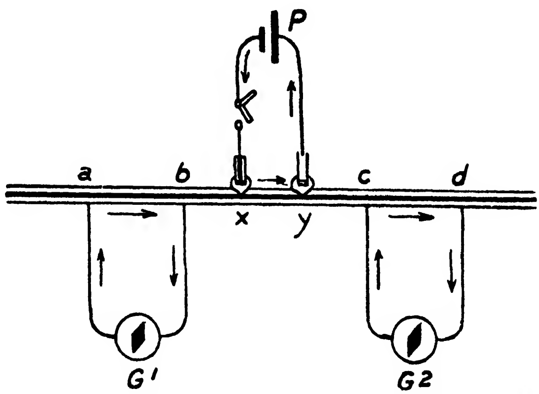


FIG. 58.—Diagram showing electrotonic currents. P, polarising circuit; G^1, G^2, galvanometers.

When the polarising circuit is closed and the polarising current passes in the nerve from x to y, the galvanometers will both indicate currents passing in the same direction, from a to b and c to d respectively. These extra-polar, or as Du Bois-Reymond called them, *electrotonic currents*, are due to the same causes as negative polarisation. Consider Fig. 59, where the positive charges are shown gathering on the surface of the axon at a-b. That is, a-b will have a higher $+$ potential than in the nerve at c or to the left of c. The result will be a flow of current from left to right

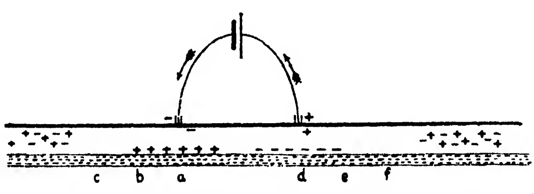


FIG. 59.—Diagram to show polarisation at the surface between conducting core and electrolyte sheath.

along the surface of the axon to reduce this difference of potential. In the same way, under the cathode at d-e the accumulation of electrons in excess of what is at f and to the right of f will cause the passage of some of the excess towards the right. It is clear, then, that all three currents, left to b (*anelectrotonic*), a to d (*polarising*), and e to right (*catelectrotonic*) flow in the same direction.

The model referred to above responds in the same way (Fig. 57),

but if a zinc wire is used as core and the cotton wool soaked in zinc sulphate to prevent polarisation, neither anelectrotonic or catelectrotonic currents are produced.

Emphasis must be placed on the fact that these electrotonic currents are absolutely distinct from the nerve impulse as well as from the wave of negativity or current of action and the current of injury. (i.) The former have a much greater velocity than the nerve impulse, as indicated by the wave of negativity. (ii.) Their E.M.F. may attain a value twenty-five times that of the current of injury. (iii.) The direction in which electrotonic currents flow depends entirely on the direction in which the primary current is flowing, reversion of the latter leading to reversion of the former. Action and injury currents always maintain a flow *in the nerve* from a stimulated or injured part to a resting or uninjured portion of the nerve.

(*b*) *Positive polarisation.* A special type of injury current, unfortunately named the *positive polarisation* current, may be obtained immediately *after* the passage of a very strong current along a nerve (Fig. 60). When the "polarising" current is broken and the anode *a* connected through a galvanometer to the cathode *k* (circuit 2), a current will be apparent flowing in the same direction as the "polarising" current, *i.e.* from *a* to *k*. This current is not due to polarisation at all. but to injury at the anode by the strong "polarising" current used,

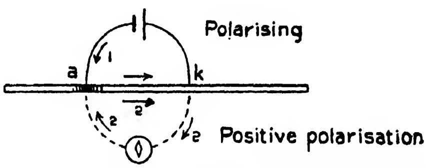


FIG. 60.—Diagram to show direction of the positive polarisation current, due to a break excitation at the anode.

A very significant series of experiments due to Lillie may be referred to here. If an iron wire is immersed in dilute nitric acid, in time it will be covered with a film of iron oxide. It is now in a passive state, and as long as the environment does not alter, no further changes will take place in or on the wire. But when the film of oxide is broken, say, by a mere scratch near one end of the wire, the break in the oxide is transmitted without decrement along the whole extent of the wire regardless of its length. This is accompanied by electrical changes and by the evolution of gas, and is followed by a restoration of the film of oxide. The wire is now passive and ready for further scratching. The model illustrates several principles of nerve action dealt with above and emphasises the point of view that the nerve impulse is associated with changes on the surface of the axon. It might repay the student to turn back to Chaps. VI. and IX., where consideration is given to the properties of monomolecular films on surfaces.

Consider again Fig. 58. Suppose that at a little to the right of d the nerve entered a muscle. With the key of circuit P open, stimulation of the nerve, say, at b by a drop of strong saline would produce convulsive twitchings of the muscle. The closure of the key completing the circuit P produces a cessation of the muscle action. The polarising current has blocked the passage of the nerve impulse. Careful experiment shows that this is due to a depression centred at x, *an anodic depression.* In a similar way, it can be shown that at y the nerve is more easily stimulated when the polarising current is running than otherwise. There is *cathodic sensitisation.* A drop of saline which, when placed at b or at c, would produce only a series of muscle twitches with the polarising current off, would cause complete tetanus at c only when the polarising current was running.

To explain this we must consider again the series of little condensers (Fig. 59). When the drop of saline acts on the nerve it produces a brief current of action, *i.e.* it tends to cause a temporary accumulation of electrons at the stimulated point and the propagation of a current of action along the nerve. When the polarising current is flowing, the condensers near x (Fig. 58) would be positively and those at y negatively charged. The action potential developed by the saline (or other stimulating cause) would, when applied in the neighbourhood of x, merely discharge the condensers, while applied at y, it would augment the charge already present leading to a more complete excitation of the structure, motor organ or sensory area supplied by the nerve.

Under experimental conditions it may be shown that the nervous impulse may be propagated in any direction in a nerve, but the nervous system *as a whole* conducts only in one direction. The unidirectional mechanism lies in the synapse, where a non-nervous substance forms the connection between neuron and neuron.

FURTHER READING

ADRIAN. " The Basis of Sensation." Christophers.

CHAPTER XIX

OUTPOSTS OF THE INTELLIGENCE SERVICE

(*a*) GENERAL AND INTRA-COMMUNAL RECEPTORS

"By mine eye, I do not know that I see, or by mine ear that I hear, but by my common sense who judgeth of sound and colours." BURTON.

IF an organism is to adapt itself to changes in the environment, there must exist within it some mechanism whereby it is "made aware" of these changes. It is as if the Cabinet and the various Local Government Boards of a country were shut up in seclusion and had to learn of the progress of the areas governed and of the various happenings abroad by messages sent in by agents from without. These agents could transmit their information by special messenger (hormones, CO_2, etc.) or by coded telegram, either to the special body controlling an area (local nerve centres), or a function (*e.g.* respiratory centre), or to the central governing body (consciousness). It is clear that a nation has to set up machinery to provide itself with two different kinds of intelligence. First it needs to know how its orders are being carried out by the civilian population as well as by the military. The internal or *interoceptive* intelligence staff is distributed among the factory workers, along lines of transport and in the various effective units of the army. Their duty is to report on the conditions in their sector. Before a shortage of raw material has become so marked as to cause an outcry from, or mayhap, a strike of some part of the population, the outposts of the intelligence staff should have their report "on the wires." The other intelligence staff operates on matters outside the organism. They are *exteroceptors*.

1. **Threshold.** The agents or receptors are specialised organs, so constructed as to have a lower threshold for one particular type of change in the environment than for all other changes. For example, the eye is specially adapted for the reception of waves in ether over a well-defined range of frequencies of quite a low intensity. It can, however, respond to other forms of stimulation, mechanical, chemical and electrical, if their intensity is sufficiently great. That is, a specialised receptor responds readily to one particular form of environmental change, even though that change

merely whispers, while all other types of change have to shout for attention.

2. Adaptation. A change in the environment of suitable nature and of sufficient intensity to be perceived by the central area concerned, ceases after a time to produce any effect. Change, ceaseless change alone, is capable of being conveyed to consciousness. The steady state is unproductive of alterations in the nervous system. We cease to be aware of the steady pressure of our clothes, of the regular ticking of the clock, of the peculiar odour of our laboratories and so on. Three structures are concerned in this: (*a*) the receptor, (*b*) the sensory nerve, and (*c*) the central sensory area. The adaptation may take place in any or in all of these. From Adrian's work we know that sensory nerves adapt themselves very rapidly and practically all at the same rate. This information is obtainable from experiments where the electrical changes that accompany the nervous impulse are made to record their own fluctuations (p. 225). When a sensory nerve fibre is stimulated electrically, only one wave of negativity passes along the nerve. Continued similar stimulation produces no further effect. Adrian dissected out an end-organ with sensory nerve intact, *e.g.* eye of eel and optic nerve, hair and cutaneous nerve, etc. He found that when an *adequate* stimulus was applied to the receptor, the electrometer, oscillograph, loud-speaker, or other electrical device, showed a burst of electrical activity lasting only a fraction of a second, even though the stimulation was continued. That is, there is a rate of adaptation for nerve plus receptor which is different from that of nerve alone. All the end-organs adapt themselves more slowly than nerves alone. Some, like those of touch and tickle, are fairly rapid, others, like pressure and muscle joint sense, are very slow. The former are termed *phasic*, and are concerned with stimuli demanding rapid action, *e.g.* touch-jump, tickle-scratch, etc. The latter are *postural*, conveying information about the position occupied by our limbs—resistance to movement, weights held, etc., not necessitating any very rapid response, but calling for the exercise of further mental processes, *e.g.* discrimination. That is, we are given time for the sensation aroused by the stimulation of a postural end-organ to "sink in." About adaptation in the sensory area, nothing can be said in the present state of knowledge.

3. Frequency of Discharge. In the previous chapter we considered the *refractory period* of nerve. This period, during which the nerve cannot transmit any propagated impulse, is, for mammalian sensory nerves, somewhat less than 1/1,000 second. That means that the nerve could conduct at least 1,000 impulses per

second if called on to do so. The receptors are not capable of such a rapid recovery. They all take about seven times as long as nerve to recover. They are, therefore, capable of transmitting as a maximum less than 150 impulses per second. This difference in time constitutes a margin of safety, *i.e.* the ingoing nerve has time completely to recover before the next impulse arrives for propagation. "*In fact*," says Adrian, "*the rapid recovery of the nerve fibre makes it practically an aperiodic conducting system as far as the slower end-organ is concerned.*" The message transmitted to the nerve by a receptor will be carried without distortion to the sensory area concerned.

4. **Stimulus and Sensation.** We cannot hope to explain on physical grounds the relationship between the nature of the stimulus and the nature of sensation (*e.g.* why ether waves of a certain frequency falling on the end-organ for sight should give us the sensation of redness, the same waves received by a heat spot arouse a feeling of warmth, etc.) until we can state in physical terms what consciousness is. We do know, however, that the *quality* (intensity and striking value) of a sensation (emotional factors being kept constant) bears a quantitative relationship to certain physical attributes of the stimulus. Adrian's technique has opened out a vast field for exploration, because consciousness can, in a sense, be eliminated. If we admit that the electrical response of a nerve is quantitatively and qualitatively a symbol of the nervous impulse, then we have an experimental tool of great importance. We can measure the stimulus in energy units and we can measure the frequency, amplitude and duration of the electrical waves induced in the sensory nerve.

Each receptor has a certain functional inertia and will not respond to stimulation until the energy of the stimulus has reached a minimal value which is specific for each receptor and for each form of stimulus. This threshold value is lowest, as has been said above, for the form of stimulation specific to that organ. Once this value is gained, the resulting sensation bears a definite relationship to the incident stimulus until an upper limiting value has been reached, after which increase of stimulation is of no avail. In fact, fatigue rapidly sets in, and the resulting sensation is submaximal.

Fechner's Law. This law states that the sensation varies as the natural logarithm of the stimulus. Adrian showed that the amplitude of movement of the mercury in the electrometer, and hence the potential difference developed in the nerve, did not vary whatever the intensity of the stimulus. The factors which do vary are the *duration of the discharge* and, in some cases, the *frequency of the discharge*. In the case of "phasic" receptors

(rapid adaptation), touch, tickle, etc., a single stimulation gives rise to the same electrical variations—frequency, amplitude and duration—irrespective of the intensity of the stimulus. A graded response can be obtained only if several end-organs are stimulated simultaneously.

To offset this lack of discriminative power the *phasic* end-organs have a very short refractory period, and so are able to accept and transmit a subsequent stimulus in a fraction of a second. Persistence of tickling, say by distortion of hairs, produces a rapid series of electrical variations. *Postural* receptors are capable of giving a graded response. We can recognise a pressure stimulus produced by 1 gram as being less than that produced by 5 grams. The movements of the mercury in the capillary electrometer indicate that, in the former case, a very brief discharge has occurred, while in the latter case, the duration of the discharge has been increased. Amplitude (*i.e.* potential difference) and frequency (*i.e.* rate of discharge) are the same for any weight giving rise to a sensation of pure pressure. Similar findings from experiments on other end-organs lead to the same conclusion, viz. *the receptor when stimulated causes an impulse to be propagated along a series of units containing ions, so that each unit becomes first negatively and then positively charged. The potential developed is a constant; the rate at which each cycle of electrical charges appears, varies from nerve to nerve (between 5 and 100 cycles per second), but is characteristic for any particular nerve fibre. The time during which the series of cycles persists is a measure of the intensity of the stimulus where adaptation is slow enough to allow of it.*

The organism is subject to stimulation from various forms of energy which may be classified into vibratory and chemical.

A. Vibratory Energy.

1. *Mechanical impacts* are received by the tactile corpuscles of the skin. They may be perceived as separate stimuli even when they arrive as rapidly as 150 per second.

2. *Slow vibrations especially in air* are received by the ear. The human ear may be stimulated by vibrations ranging from 16 to 40,000 per second. Practice may extend this range.

3. *Rapid vibrations in ether.*

(*a*) *Radiant heat.* Vibrations with a frequency of between 3 billions and 400 billions per second stimulate the temperature receptors of the skin.

(*b*) *Light.* The retina is capable of receiving as light, ether waves, the frequency of which varies between about 400 billions and 800 billions per second.

B. Chemical Energy.

The various chemical stimuli to which the organism is exposed have receptors in the skin, giving rise to sensations of pain or discomfort, and in the special end-organs to those of taste and smell.

As receptors for these various manifestations of energy we have the so-called five senses. That is, five different means are employed for the purpose of orientation, viz. touch, hearing, sight, smell and taste. These senses come into contact with the external forces through the skin, ear, eye, nose and tongue. But some of these are composite end-organs. The skin, for instance, includes not only touch corpuscles but the end-organs for pain and temperature. The ear not only analyses sounds, but contains organs for the static and dynamic senses. In all there are over twenty different kinds of receptors and sense-organs in the body.

I. PHASIC RECEPTORS

1. Touch is the sense by which mechanical force is appreciated. Mere contact is gentle pressure, a greater amount of applied force causes a feeling of resistance referred to the skin, a still greater amount evokes a response from receptors in the muscle, while pain results from great pressure. The total number of tactile corpuscles (excluding those on the head) has been estimated as 500,000. These are not evenly distributed over the skin, but are more numerous and more sensitive on certain of the more mobile parts of the body, *e.g.* tongue and fingers. The degree of sensitiveness of the skin may be determined by some form of aesthesiometer (say a pair of compasses) by means of which one may measure the smallest distance at which impress of the two points may be perceived as two distinct sensations. The following table gives the activity of the discriminating sense for different parts of the skin :

TABLE XXXIII.

	Millimetres.
Tip of the tongue	1·1
Third phalanx of finger, volar surface	2–2·3
Red part of the lip	4·5
Second phalanx of finger, volar surface	4–4·5
First phalanx of finger, volar surface	5–5·5
Third phalanx of finger, dorsal surface	6·8
Tip of nose	6·8
Head of metacarpal bone, volar surface	5–6·8
Ball of thumb	5·7–6
Ball of little finger	5·5–6

TABLE XXXIII—*continued.*

	Millimetres.
Centre of palm	8–9
Dorsum and side of tongue; white of lips; metacarpal part of the thumb	9
Third phalanx of the great toe, plantar surface	11·3
Second phalanx of the fingers, dorsal surface	11·3
Back	11·3
Eyelid	11·3
Centre of hard palate	13·5
Lower third of the forearm, volar surface	15
In front of the zygoma	15·8
Plantar surface of the great toe	15·8
Inner surface of the lip	20·3
Behind the zygoma	22·6
Forehead	22·6
Occiput	27·1
Back of the hand	29·8
Under the chin	33·8
Vertex	33·8
Knee	36·1
Sacrum, gluteal region	44·6
Forearm and leg	45·1
Neck	54·1
Back of the fifth dorsal vertebra; lower dorsal and lumbar region	54·1
Upper arm; thigh; centre of back	67·7
Middle of the neck	67·7

The intensity of the contact sensation is increased in a mechanical way by the presence of hairs, because they act as levers on the tactile corpuscles. The whiskers of the cat render the touch points of the jaw very sensitive in this way, being able to detect even slight air currents.

2. **Tickle** is a sensation that may be classed among the surface phenomena like touch, or among the deeper sensibilities like pressure. Dealing exclusively with the former variety, we may say that the stimuli producing it are light, intermittent or stroking touches applied to the surface of the body. We must again distinguish between stimulation of a surface furnished with hairs, such as the back of the hand, nape of the neck, etc., and those surfaces that are hairless, such as the sole of the foot, dorsum of the tongue, back of the throat, etc. In the former case, even a gentle touch applied to the end of a hair, *provided it is sufficient to bend the hair*, gives rise to a short burst of electrical waves along the sensory nerve, and adaptation occurs rapidly, *i.e.* the receptor is of the *phasic* type. Histological examination shows that the hair acts as a lever transmitting pressure to the tissue surrounding its root, in which are embedded arborisations of sensory nerve

fibres. The hairless surfaces when touched only give rise to a sensation of tickle when the touch is intermittent or stroking. It need not necessarily be light, *e.g.* the sole of the foot may be tickled intensely by rubbing vigorously with a hard nail brush (Greig). The receptors for this type of stimulation are also phasic, and depend on pressure in the sublying tissues (Fig. 61).

The muscles at certain places are extremely sensitive to inter-

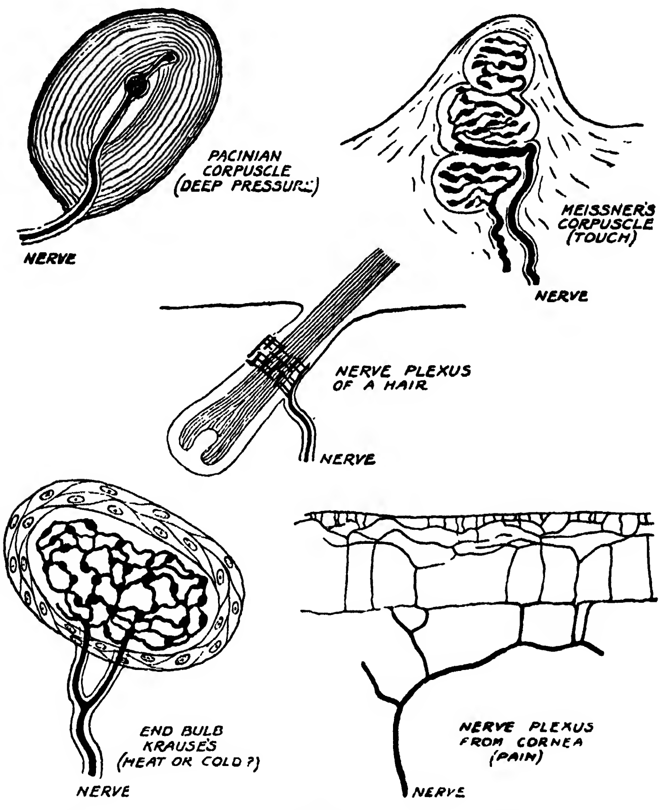


FIG. 61.—Various types of receptors found at or near the surface of the body.

mittent pressure, *e.g.* at ribs and knees. Here the stimulus is distinct pressure, and is allied with the sense of pain. Continued tickling has been used as a means of torture by Eastern peoples. Simon de Montfort is said to have put the Albigenses to death by tickling, and a certain Anabaptist sect, unwilling to shed blood, are reported to have used this method of executing offenders.

3. Pain is aroused by mechanical, thermal or chemical stimuli of sufficient intensity, and is considered by some to be caused by overstimulation of any receptor, *e.g.* too loud a sound, too bright a

light, too hot or too cold an object touching the body. Recent work, however, leads one to the conclusion that although abnormally intense stimuli may cause a painful sensation, there are specific receptors for pain. For example, pain spots may be demonstrated in the skin, in much the same way as touch spots, using a needle in place of a soft bristle. Further, pain may be elicited by the stimulation of surfaces known to contain no other receptors, *e.g.* cornea. The electrical response on stimulation of a pain spot is of similar frequency (5–100 a second) to that produced by touch. It differs in its duration and intensity. A slight pain would be one accompanied by an electrical discharge lasting, say, 1 second, while the discharge during acute pain may last about 20 seconds. Touch produces an electrical burst lasting about 0·2 second. It may be that a brief discharge passing along a nerve fibre is the sign of the passage of a nervous impulse producing a change in consciousness which we have learned to associate with touch, while a longer discharge *in the same fibre* signifies pain. This is not true of all touch receptors. For instance, Meissner's corpuscles never give rise to pain when stimulated. The naked terminations of nerve fibres arborising in the skin may, as Sherrington suggests, act as receptors for touch and pain—touch when the stimulus is slight, and pain when it is massive.

Pain may be produced by direct stimulation of the nervous system, either by the application of induction shocks, by mechanical force (pulling, tearing, pressing, etc.), or by chemical means (drying, application of solutions, etc.), and in this case the intensity of the pain depends on the *number* of nerve fibres in the nerve trunk stimulated. The central sensory area may also receive painful stimulation without the apparent intervention of specialised receptors. A rise in blood pressure, for example, results in a headache relieved by the administration of a vasodilator. Pain, then, may be considered as allied to both touch and pressure, but possessing the characteristic of a massiveness in the electrical variations accompanying its transmission to the cortex.

II. PHASIC-POSTURAL RECEPTORS

4. **Pressure.** The receptors for pressure are more of the ***postural*** than of the ***phasic*** type. That is, a single continued stimulation produces a prolonged sensation. In fact, the sensation outlasts the stimulus by quite an appreciable time. Absolute sensitiveness as indicated by a sense of pressure is generally determined by finding a minimum pressure necessary to evoke a minimal sensa-

special end-organs at the tip of the tongue, if it contains both a gluciphoric and auxogluc grouping. The classification is not quite satisfactory. Progress will be made when the problem is attacked by physical chemists from the aspect of permeability, much in the same way as we shall see has been done for sour tastes.

Sourness. This sensation is produced when acids penetrate certain cells on the sides of the tongue. The threshold value for sourness does not depend on the *strength* of the acid alone. For example, acetic acid, a weak acid, is able to affect the cells at a H ion concentration less than that necessary for strong acids like HCl, HNO_3, etc. This is probably due to the greater penetrating power of the weaker acid in an undissociated state. It then dissociates in the cell, liberating H ions to act on the end-organs. In the following table, XXXVI., is given a list of some organic acids with their minimum concentration just to be appreciated as sour, *i.e.* threshold value (from Taylor), and with the concentration gradient necessary to produce a *p*H of 5·6 in *Chromodoris* tissue in 20 minutes (from Crozier, 1916).

TABLE XXXVI.

Acid.	Threshold Value for Taste.		Penetration.	Concentration.
	Concentration $\times 10^{-4}$ N	H^+ Concentration $\times 10^{-4}$ N	Concentration. $\times 10^{-4}$ N	H^+ Concentration $\times 10^{-4}$ N
Formic . .	18	5·5	33	7·5
Oxalic . . .	20	11·6	10	9·5
Tartaric . .	22	7·0	33	10·5
Acetic . . .	28	2·8	188	5·8
Lactic . . .	28	11·7	52	7·9
Succinic . .	32	3·4	42	3·6
Butyric . .	35	2·7	93	3·6

It will be seen from this table (1) that weak acids, like acetic, butyric and succinic, are effective at a remarkably low H^+ concentration, both in causing taste and in penetrating tissue; and (2) that the introduction of a hydroxy group generally (not always) decreases the power of stimulation and penetration, *e.g.* lactic acid (hydroxy-propionic) is about half as active as propionic acid, and salicylic about one-third as penetrating as benzoic acid. Carbonic acid, which may be considered as hydroxy-formic acid, apparently is an exception to this generalisation, as it is about fifty times as active as the fatty acid. This is probably due to its passage into the tissues not as H_2CO_3, but as CO_2, which is soluble both in water and in fats.

That only four kinds of taste can be recognised is readily understood when consideration is given to the four types of receptors, each with a lower threshold for one particular form of chemical stimulation. Some substances are able to stimulate more than one group of gustatory end-organs. For example, saccharine penetrates and stimulates the receptors at the back as well as those at the tip of the tongue. It is, therefore, perceived as both bitter and sweet. In the same way, acetate of lead is sweet and sour; acetone, sweet and bitter; potassium sulphate, bitter and sour; magnesium chloride, bitter and salt, and so on. Then other tasty solutions are mixtures of substances, each stimulating at a different part of the tongue, *i.e.* a compound stimulation. Proof of this fourfold taste complex has been obtained in man by stimulating the afferent nerve fibres for taste in the *chorda tympani* of an individual with a fistula in his ear. Trials were made at different times and by various direct means of stimulating the nerve, and, in every case, the subject reported sensations of sweetness, sourness, bitterness or saltness only. One must conclude, in view of Adrian's work, that we differentiate between tastes not so much because a different stimulus is applied, but because it is applied *at a different place*, *i.e.* a *primary* analysis takes place at the end-organ. As far as one can judge, the nature of the nervous impulse is the same for all tastes and, therefore, *final* analysis must take place in the sensory centre. That is, we appreciate the difference between sweet and bitter, say, in the same way as we differentiate between a touch on the arm and a similar stimulus applied to the calf of the leg.

7. **Smell.** The sense of smell added to that of taste contributes in large measure to the pleasures of the table and serves as an excellent substitute-stimulus for the flow of saliva and gastric juice (conditioned reflex). Flavours, as a matter of fact, are olfactory and not gustatory stimulants. If we lose our sense of smell, say by a cold in the nose or by experimentally preventing the entrance of gases into the upper nasal passage, much of our food seems to become tasteless. We are unable in these circumstances to tell whether we are chewing raw potato or raw apple.

Smell is the ancestral chemical sense and may be classed, especially in the lower animals, as a distance receptor. In civilised man this sense, unless rendered acute by training, is merely vestigial.

The areas of nasal mucosa associated with this perceptive mechanism are small rectangular strips in the upper part of each nasal cavity, just above the superior turbinate bone. In ordinary respiration, air does not pass directly over the olfactory mucous

membrane, but some air diffuses backways through the posterior nares (Fig. 62, upper portion). This is important for the preservation of the sense. The receptor neurons have retained their primitive condition of cell body in the epithelium itself (Parker). They are rapidly fatigued and readily destroyed. Now, by their situation in a backwater they do not come directly into contact

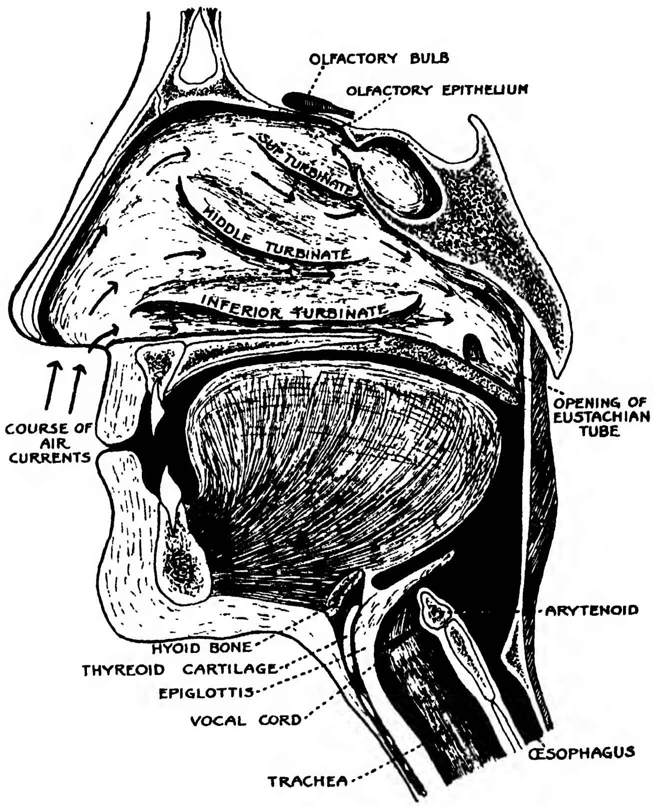


FIG. 62.—Diagram of antero-posterior section through nasal fossæ, mouth and neck. In the upper portion of the figure the arrows show the direction of the air currents during inspiration. The soft palate should, of course, be down to allow of the passage of air. The lower portion of the diagram represents the position of the structures during the act of swallowing.

with high concentrations of odoriferous substances and, furthermore, air attains body temperature and moisture, and is freed from suspended particles (dust, bacteria, etc.) before reaching the sensory surface. The physical details of the mechanism for the perception of smell, that is, for the conversion of chemical into nervous energy, have not yet been brought to light. The sense is extraordinarily delicate. Mercaptan, in as low a concentration as 0·0000000004 gram per litre of air, can be detected. Training renders the sense more acute. The working chemist relies on his sense of smell to

a great extent to help him in the identification of compounds. The tea blender and the wine expert can detect very slight differences in " flavour."

It is worth while noticing that receptors all depend for stimulation on the existence of an *alteration* in external energy. This is specially marked in the case of this ancestral chemical sense. Our accustomed environment presents no stimulus. Air has no smell and water no taste. The introduction of a trace of foreign body alters the energy content of the environment and stimulation follows. It is a common experience to find that people do not experience sensations which have, for the time being, become permanent in their environment. A room may be stuffy to an incomer but quite comfortable to the tenants. The physiological chemist works in an atmosphere which causes his visitor to choke and splutter, but the introduction of a new odour, say ammonia, is at once perceived and produces instant action.

The primary odours are (i.) flowery (violet), (ii.) fruity (lemon), (iii.) spicy (nutmeg), (iv.) resinous (frankincense), (v.) putrid (H_2S), and (vi.) burning (tar). Other olfactory sensations are mixtures of two or more of these, *e.g.* vanilla = (i.) + (ii.), garlic = (ii.) + (v.), and so on.

How do these substances produce a stimulation of the olfactory epithelium ? The present idea is that in the gaseous state they produce a series of waves in ether, part of the electro-magnetic spectrum, having frequencies much greater than those of light. This is quite plausible, as odorous substances belong almost exclusively to the fifth, sixth and seventh groups of the periodic classification, in which the elements are characterised by the possession of variable valences, *i.e.* can set free electrons. The " strength " of a smell appears to be related to the speed of rotation of the valence electrons (Chap. XIII.). It is interesting to note that ultra-violet light, which is known to have the property of stabilising these substances by destroying the double bonds in them, also destroys their odour.

8. **Hunger** is a sensation which must be regarded as primitive and basal. It is not our business to analyse the feelings of hunger, but to consider the mechanism by which the lack of nourishment is signalled to consciousness. The evolution of knowledge of this sensation is largely due to Cannon, whose book on the subject should be read by every student. There can be no doubt that the feeling of hunger is closely allied to pain.

" The sensation of hunger is difficult to describe, but almost everyone from childhood has felt that dull ache or gnawing referred to the lower mid-chest region or epigastrium, which takes

imperious control of human actions. As Sternberg has pointed out, hunger may be sufficiently insistent to force the taking of food which is so distasteful that it not only fails to rouse appetite but may even produce nausea. The hungry being gulps his food with a rush. The pleasures of appetite are not for him—he wants quantity rather than quality, and he wants it at once.

Hunger may be described as having a central core and certain more or less variable accessories. The peculiar dull ache of hungriness referred to the epigastrium is usually the organism's first strong demand for food; and when the initial order is not obeyed, the sensation is likely to grow into a highly uncomfortable pain or gnawing, less definitely localised as it becomes more intense. This may be regarded as the essential feature of hunger. Besides the dull ache, however, lassitude and drowsiness may appear, or faintness, or violent headache, or irritability and restlessness such that continuous effort in ordinary affairs becomes increasingly difficult. That these states differ much with individuals—headache in one and faintness in another, for example—indicates that they do not indicate the central fact of hunger, but are more or less inconstant accompaniments. The 'feeling of emptiness,' which has been mentioned as an important element of the experience, is an inference rather than a distinct datum of consciousness and can likewise be eliminated from further consideration. The dull pressing sensation is left, therefore, as the constant characteristic, the central fact to be examined in detail" (Cannon).

Cannon and his colleagues have definitely proved that the sensation of hunger is caused by strong contractions of parts of the alimentary canal. As we shall see later when dealing with transport (Chap. XXVIII.), there are certain definite movements of the alimentary canal designated as peristaltic, associated with the forward transference of the contents of the canal. In the absence of any content other than gaseous, the cavities of the stomach, lower œsophagus and upper intestinal region, at least, are almost obliterated. This wave of contraction precedes the sensation of hunger and may be regarded as the cause of it. Carlson and his students, who were fortunate in having a subject with a permanent gastric fistula, have confirmed Cannon's work and carried it further. They have shown that the local contraction is a sign of a general state. According to Carlson and Luckhardt the blood of a fasting animal, if injected into the vein of a normal animal, is capable of producing in the latter contraction of the gastric muscles, an effect which does not occur when the blood of a well-fed animal is injected.

The significance of this phenomenon is plain. In Cannon's words:

"The very condition which causes hunger and leads to the taking of food is the condition, when the swallowed food stretches the shortened muscles, for immediate starting of gastric peristalsis. In this connection, the observations of Haudek and Stigler are probably significant. They found that the stomach discharges its contents more rapidly if food is eaten in hunger than if not so eaten. Hunger, in other words, is normally the signal that the stomach is contracted for action; the unpleasantness of hunger leads to eating, eating starts gastric digestion and abolishes the sensation. Meanwhile the pancreatic and intestinal juices as well as bile have been prepared in the duodenum to receive the oncoming chyme. The periodic activity of the alimentary canal in fasting, therefore, is not solely the source of hunger pangs, but is at the same time an exhibition in the digestive organs of readiness for prompt attack on the food swallowed by the hungry animal."

Further Reading

Adrian. "The Basis of Sensation." Christophers.
Cannon. "Bodily Changes in Pain, Hunger, Fear and Rage." Appleton
Harris. "The Functional Inertia of Living Matter." J. & A. Churchill

CHAPTER XX

OUTPOSTS OF THE INTELLIGENCE SERVICE

(b) DISTANCE RECEPTOR FOR SOUND

THE EAR

"A clue to the structure of a machine lies in the discovery of the purpose for which it was designed and the manner in which its various parts are co-ordinated to secure that end. That is eminently true of the ear." KEITH.

THE ear is a modified touch receptor. In the lower invertebrates it consists of hair-like appendages, either on the free surface or in a depression, more or less protected. In the higher vertebrates it is a much more complicated structure. The human organ of hearing may be considered as composed of three structural elements, viz:

External ear—collector and conductor of sound to the middle ear.

Middle ear—converter of air vibrations to a to-and-fro movement of a hinged piston-like lever and the accentuation of these movements.

Part of internal ear—transformer of mechanical pressure, *viâ* hydraulic pressure, into touch stimulus.

1. **External ear.** The structure of this presents no outstanding points of physical interest. It consists of the *pinna* and the *external acoustic meatus* at the end of which is the *membrana tympani* or eardrum (Fig. 68).

(*a*) **The pinna** is a flattened horn presenting irregularities of surface. If these undulations are filled in with wax or if the pinna is awanting, the quality of sounds is altered and difficulty in localising sound is increased. This may be due to a differential reflection of tones by the pinna, *e.g.* it may reflect a fundamental tone more strongly than the partial or *vice versâ*.

(*b*) **The external acoustic meatus** is a curved tube about 21–26 mm. long. Its function is twofold. (i.) On account of its shape, secretion, and hairs (at orifice) it protects the delicate tympanic membrane from draughts, dust, and from the incursion of insects, and maintains an equable temperature. These are its main functions. (ii.) The sound waves are conducted by reflection from the walls without loss of intensity, and directed almost perpendicularly on to the drum which lies at an angle of 150° to the axis of the canal.

2. Middle ear. The mechanism found in the middle ear converts vibrations in air into vibrations in fluid by means of membranes and a series of levers. It consists of an air-filled cavity hollowed out of the petrous part of the temporal bone. It is separated from the external ear by the tympanic membrane, and from the internal ear by the membrane closing the round window and by a disc of bone—the foot of the *stapes*, which along with the membranous collar surrounding this bone makes a fluid-tight packing or gland

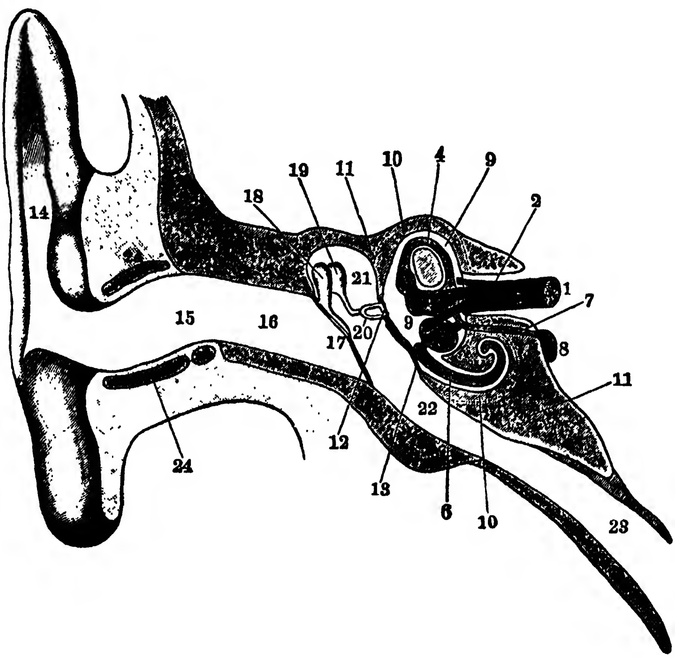


FIG. 63.—Diagrammatic view of auditory organ. (After Schafer.)
1, Auditory nerve; 2, internal acoustic meatus; 3, utricle; 4, semicircular duct; 5, saccule; 6, scala media of cochlea; 9, vestibule containing lymph; 10, semicircular canal; 12, stapes; 13, fenestra rotunda; 19, incus; 18, malleus; 17, membrana tympani; 16, external acoustic meatus; 14, auricle or pinna; 23, Eustachian tube.

filling the *foramen ovalis*, the oval opening into the internal ear. Between the drum and the stapes lie two bony levers—the *malleus* and the *incus*.

(*a*) **Membrana tympani.** This structure is fixed in a frame of bone which is almost circular (vertical diameter 10 mm.; horizontal diameter 8·5 mm.). Although it is not more than 0·1 mm. thick, it is constructed of three layers. On the outer surface there is a layer of epithelium protecting the membrane proper, which is of fibrous tissue and is covered on the inner side by a layer of mucous membrane. The fibres of the fibrous layer are arranged partly circularly and partly radially—the circular fibres

being most marked near the rim. To the inner surface is attached the handle of the *malleus*, the first of the chain of three auditory ossicles. This attachment to the *malleus*, which is pulled inwards by the *tensor tympani* muscle, gives the tympanic membrane the form of an eccentric funnel opening outwards. The membrane is highly elastic and responds very readily to very slight variations in the pressure of the air waves entering the external ear. The peculiar form of the membrane contributes to its value as a sound transmitter. In the first place it acts *synkinetically, i.e. moves passively with* the vibrations of the sound-waves. It begins and ends its vibrations synchronously with the impact of the sound vibrations. There is no latent period, no waiting for a summation of impulses before it can get into its swing, having no swing to get into. It does not continue to vibrate after the sound vibrations have ceased. It is dead-beat. Further, it does not vibrate sympathetically to any special overtone present in a compound tone reaching the ear. This is brought about by (i.) the damping effect of attachment to the ossicles and (ii.) by the dragging inwards at the point of attachment (umbo). On this account the fibres vary in tension as well as in length, so endowing each bit of the membrane with a different period of vibration resulting, *in toto*, in an aperiodic membrane. It is obvious that such a property is valuable in rendering hearing distinct. In the second place the arched sides of the membrane act as a lever of the 1st class.

"As the outward curvature of the radial fibres is slight, each fibre may be regarded as the long arm of a lever, while the handle of the hammer is the short arm. This mechanism secures that a slight pressure of the air corresponding to a sound wave, exerts a considerable force upon the malleus. To aid in understanding the mechanism, it will be easier to consider, first, the effect of pressure upon a single radial fibre. The fibre may be regarded as inextensible and slightly curved outwards; hence variations in pressure on the convexity of the curve will cause the degree of curvature to change, while the length of the arc will remain the same. In other words, the radius of the arc and the chord of the arc will change, while the length of the arc remains constant."

Hence it may be shown that "the greater the radius of curvature, the greater will be the alterations in tension of the fibre caused by alterations in the pressure of the air. Further, as the radial fibres are those which are attached to the malleus, it is evident that the variations in the tension of the fibres cause movements of the bones when sound-waves strike the drum-head. Thus a very small change of pressure in the air causes a considerable change in the tension of the fibres; and further, in accordance with the laws

regulating the action of the lever, as the force which fibres exert upon the handle of the malleus increases, amplitude of movement of that bone diminishes. In this way, the special form of the drum-head secures a maximum of efficiency for tones of the feeblest intensity " (M'Kendrick).

Briefly, energy applied to the membrane is passed on to the handle of the malleus diminished in amplitude but with increased intensity.

(*b*) Ossicles. The three bones of the middle ear—the *malleus*, the *incus* and the *stapes*—stretch across the tympanic cavity forming an articulated chain of levers, so that every normal movement of the tympanic membrane is transmitted by the *stapes* to

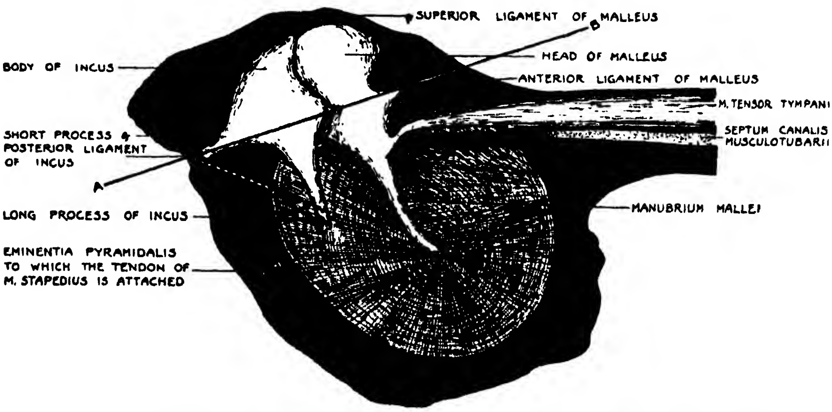


FIG. 64.—Diagram of the left Membrana Tympani and Chain of Tympanic Ossicles seen from the medial aspect.

The line *A*—*B* is the axis of rotation of the malleus and incus. The dotted line represents the line of leverage applied from the handle of the malleus to the posterior ligament of the incus. The stapes lies almost at right angles to the plane of the paper.

the fluid of the internal ear. The Malleus, or hammer, is about 18–19 mm. long, and has an average weight of 23 mg. It consists of a thickened rounded head and a long handle—the *manubrium*, which is attached to the tympanic membrane, the tip of the handle reaching the umbo. Near the insertion of the head and handle arises a bony process—the *processus gracilis* or *processus Folianus*, which projects forward and is continued by a ligament, the *anterior ligament* by means of which the hammer is anchored to the wall of the tympanum. There is also a shorter protuberance, the *processus brevis*, which presses against the edge of the upper surface of the drum. Three other ligaments are attached to the malleus, the *external ligament*, binding it to the external face of the tympanic cavity, the *superior or suspensory ligament*, attaching the top of the head to the roof of the cavity, and the posterior ligament. These ligaments prevent the *malleus* from

rotating on any other axis than a horizontal one whose line passes through the head of the *malleus* and the *anterior ligament* (Fig. 64). The posterior surface of the head of the hammer fits into the saddle-like hollow in the anterior surface of the body of the anvil-bone or *incus*. This is a larger bone than the *malleus*, weighing on the average 25 mg. The body of the *incus* is drawn out on its posterior side to a process—the short process, which is attached by a ligament to the posterior wall of the tympanic cavity, forming one end of the malleo-incal axis mentioned above. Almost at right angles to the short process, the inferior surface of the *incus* tapers down to the knob-like *os orbiculare*, forming the long process.

The *os orbiculare* articulates with the knob on the top of the stirrup bone or **stapes**. This bone, a flattened stirrup arch, weighing only about 3 mg., is set almost at right angles to the long process of the incus. Its oval footplate is attached to the margin of the *fenestra ovalis* by a short stiff membrane, the *annular ligament*.

Muscles of middle ear.

Two slender muscles are attached to the ossicles :

(i.) The **stapedius** is inserted into the knob at the head of the stapes and is attached to the posterior wall of the tympanic cavity.

(ii.) The **tensor tympani** arises from the inner wall of the cavity, passes outwards and upwards above the *Eustachian tube*, to be inserted in the upper part of the handle of the *malleus*.

Function of the muscles.

The tensor, on contraction, draws the handle of the *malleus* inwards, and so, as its name implies, increases the tension on the tympanic membrane. This decreases the natural period of vibration of the drum, and this makes it more sensitive to high tones, and better fitted to adjust its vibrations to rapid changes of phase. Paralysis of this muscle impairs hearing.

The *stapedius* prevents the footplate of the *stapes* from having purely a piston-like action on the *fenestra ovalis*. Its line of traction (Fig. 64), which is almost parallel to the long axis of the oval window, causes the footplate to move on the posterior annular ligament as on a hinge. Contraction of this muscle thus draws the anterior end of the footplate outwards.

These two muscles are therefore antagonistic ; simultaneous contraction balances the ossicles and regulates the degree to which the perilymph of the internal ear is displaced. Further, the tension of the two muscles prevents a slack engagement

between the ossicles. If the bearings were not kept together with sufficient force, slipping, knocking and loss of power would ensue. This state of equilibrium is absolutely necessary if the system of membranes and ossicles is to move in immediate response to the slightest alteration in air pressure.

Before going into the mode of action of the ear bones, a pressure equalising device comes up for consideration. As has just been said, perfect equilibrium of vibrating parts is necessary for perfect transmission of energy. One can therefore realise how important it is for there to be some open communication between the tympanic cavity and the atmosphere. By means of the **Eustachian tube**, communication is established between the middle ear and the pharynx and through the latter with the exterior, and so both sides of the tympanic membrane are kept at atmospheric pressure. Normally, it is closed by an arch of cartilage which surrounds the lower end. The *tensor palati* muscle is inserted in one side of this arch, and when this muscle contracts during the act of swallowing (Chap. XXVIII.), it draws down and flattens the cartilage and so opens the tube. Occlusion of the tube by mucus or by inflammation of the throat isolates the air in the middle ear. The air is gradually absorbed by the tissues, pressure is thus reduced, and the drum is sucked inwards. This increased tension in the membrane makes it less responsive to sound. Temporary deafness caused by a rapid alteration in external air pressure (*e.g.* rising in an aeroplane ; descending in a submarine, or diving in a bell or suit, entering a caisson, etc.) is immediately relieved by movements of swallowing. The Eustachian tube is also the drainage tube to the middle ear, preventing the accumulation of mucus (Figs. 62 and 63).

Mechanism of the middle ear.

The function of the mechanism is to transform the alternate condensations and rarefactions of air, which we call sound, into a series of hydraulic movements of the fluid in the internal ear. Direct observation has shown that the ear-bones form a chain of levers which together conduct the vibrations incident on the drum to the foot of the *stapes*.

A. Let us look first at the **mechanism of the levers.** In Fig. 64 is given a schematic sketch of the ossicles illustrating their lever action. The axis on which the *malleus* and *incus* together turn is represented by the line *A—B* passing from the tip of the short process of the *malleus* to the tip of the short process of the *incus*. A movement inwards of the *manubrium* will cause the head of the *malleus* to swing outwards, carrying with it the upper part of the *incus* and so moving the long process of the *incus* in the same

direction as the *manubrium*. This movement is directly transmitted to the *stapes*. The chain of bones, therefore, acts as a bent lever whose fulcrum is at **a**,* the power arm being represented by the dotted line and the load arm by the line **i**—**a**. According to Helmholtz, the distance **p**—**a** is 9·5 mm., and **i**—**a** is 6·3 mm. The movement at **i** therefore will be only two-thirds of the movement at **p**, but will have $1\frac{1}{2}$ times the intensity. We have now to consider the transmission of this power to the foot of the stapes. If x—y represents the range of the tip of the *incus*, c—d the distance from c, the hinge (lower annular ligament) to d the centre of the foot of the stapes, and c—i the distance from the tip of the long process of the incus to the lower end of the stapes, then the range of motion of the centre of the foot of the stapes will be $\frac{(x-y \times (c-d)}{(c-i)}$ which, according to the scale of a drawing given by Helmholtz, would give a leverage of about 2—1.

So that, on the whole system of bones there is a leverage of about 3—1. This theoretical value is, however, reduced by the friction of the levers and by the damping effect of the air filling the internal ear. It has been estimated that half the force is thus dissipated.

Three further points about the chain of ossicles claim our attention. Firstly, by the position of the axis, *A*—*B*, the mass of the heads of the hammer- and anvil-bones are above the line, while the lever arms are below the line on which the bones rotate. This keeps these two bones suspended in equilibrium. Secondly, there are tooth-like processes on the surface of the hammer which engage with the body of the anvil, enabling each to move the other in the to-and-fro movements of the drum. In the case of unusually sudden alterations in air-pressure, *e.g.* a blow on the ear, these processes slip over each other and prevent damage to the internal ear. Thirdly, by the way in which they are hung on opposing ligaments, and controlled by opposing muscles, they form a kind of balance wheel which is very sensitive to the transmission of power in small vibrations. Its efficiency in this respect is derived from the fact that the elastic forces balance one another in the mechanical centre of the system, and so practically the whole power applied to the drum is transmitted to the foot of the stapes.

B. The pressure in the internal ear is reinforced not only by the system of lever transmission but by the relative sizes of the membranes at either end of the chain of ossicles. The area of

* **a** = junction of dotted line with the axis *A*—*B*; **i** = tip of long process of incus; **p** = point of application of power, *i.e.* tip of manubrium mallei (Fig. 64).

the tympanic membrane is about twenty times the area of the *fenestra ovalis*. This means that, keeping the total power constant, the power per unit area is increased twenty times. This is augmented by the intermediate leverage (correcting for air-damping, friction, etc.), which we have seen has been estimated as *not less than* $1\frac{1}{2}$—1. This would give a total increase of effective pressure of *at least* 30—1. (Wrightson puts the value as high as 60—1 on the assumption that no slip occurs at the malleo-incal joint, etc.)

3. **Internal ear.** The internal ear is a somewhat complex cavity in the petrous part of the temporal bone. Two separate organs are housed in this cavity, viz. the labyrinth by which equilibrium is maintained, and the cochlea.

The Cochlea is a tube, 20–30 mm. long, which takes two and a half spiral turns round a conical bone, the *modiolus* through the centre of which the auditory nerve passes. The cochlea is divided into three portions by means of (*a*) a spiral lamina of bone extending from the *modiolus* about two-thirds across the tube, and (*b*) joined to the walls of the tube by two membranes, Reissner's and the basilar membrane. The former is a thin layer of cells and separates the vestibular duct from the intramembranous middle duct. The part below the basilar membrane is called the tympanic duct. The *fenestra ovalis* closes the vestibule—the swelling at the wide end of the *scala vestibuli*—while the *fenestra rotunda* does similar service to the lower duct, the *scala tympani*. The two ducts are united at the apex of the cochlea by an irregular crescentic aperture called the **helicotrema**. This opening has an average area of 0·15 sq. mm.—markedly less than the sectional area of the terminal part of either scala. These scalae are filled with a fluid, **perilymph**, which obviously, because of the *fenestra rotunda*, is normally under atmospheric pressure.

Chief interest in the internal ear lies in the structure of the **scala media** and its contents (Fig. 65). It is triangular in section, having for base the basilar membrane which separates it from the tympanic duct; the long side is composed of *Reissner's membrane*, which divides it from the vestibular duct. The short side is separated from the outer wall of the osseous cochlea by a vascular layer (*stria vascularis*), laid on and in a continuation of Reissner's membrane, which in turn is placed on the spiral ligament, or pad. Roughly, the cubic capacity of this duct is about a quarter of that of the *scala tympani* and about a third of that of the *scala vestibuli*. It is filled with *endolymph*, a fluid similar to the perilymph of the rest of the cochlea. No communication exists between the scala media and any other part of the *cochlea*. There

is a narrow tube, the *canalis reuniens*, which runs from this duct to the saccule—part of the organ for maintaining equilibrium.

The reason for the attention that has been directed to the scala media is that the **cochlear division of the auditory nerve**, which runs down the *modiolus*, enters only this scala, passing along the basilar membrane and ending in dendrites among the hair-cells of the **organ of Corti**. This structure is a development of the epithelium lining the tube. It is set on the basilar membrane at its junction with the *limbus laminae spiralis*, and consists of four essential

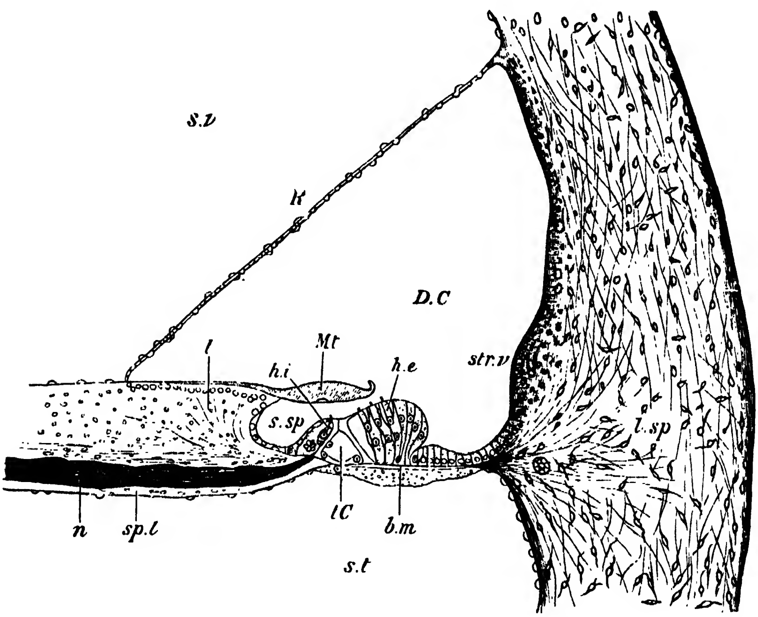


FIG. 65.—Vertical section of the first turn of the human cochlea (G. Retzius).
s.v, scala vestibuli; *s.t*, scala tympani; *D.C*, scala media; *sp.l*, spiral lamina; *n*, nerve fibres; *l.sp*, spiral ligament; *str.v*, stria vascularis; *m.t*, membrana tectoria; *b.m.*, basilar membrane; *h.i*, and *h.e*, internal and external hair cells; *R*, section of Reissner's membrane; *t.C*, tunnel of Corti; *l*, limbus laminae spiralis.

elements. (1) Certain columnar cells with short stiff hair-like processes projecting from their free border, the **hair cells**, to which pass, as we have just said, branches of the cochlear nerve; (2) elongated strengthening cells between the hair cells, **cells of Deiters**, the peripheral processes of which join together to form a network through which the hair-cells project (*membrana reticularis*); (3) stiff short fibres set one against another in the form of an arch; and (4) an exceedingly delicate membrane attached to the upper surface of the spiral lamina, and lying over or fixed to both the outer and the inner walls of Corti's organ (*membrana tectoria*).

The arch of Corti, which lies just outside the single row of inner

hair cells, is composed of a row of inner "rods," shaped like ulnar bones, attached by their terminal end to the basilar membrane, and fitting on to the heads of the outer "rods." The latter resemble swan's heads and necks, the backs of the heads fitting into the hollows of the inner "rods."

The mechanism of the internal ear.

It is obvious that every movement of the *stapes* is communicated by the *perilymph* to the membranes of the *scala media,* from them to the *endolymph,* and so to the *organ of Corti.* There is also no doubt about the hair cells as being the final instruments for the transmission of the impulses to the nerve. The only structures placed so that their movements can be transmitted to these hair cells are (*a*) the basilar membrane, (*b*) the tectorial membrane, and (*c*) the pillars of the arches of Corti. We may dismiss Corti's arches as the basis whereby fluid motion is converted into the movements of the hairs, because the cochlea of birds is free from them. Opinion is sharply divided as to the comparative importance of the two membranes. Because of its structure, position and marked differentiation at different levels, most modern investigators are of the opinion that the basilar membrane plays the more important part.

Several theories have been put forward as to the mechanism of the inner ear. The most important are (i.) the resonance theory associated with the names of Helmholtz, McKendrick, Hartridge and Wilkinson; (ii.) the displacement theory of Wrightson and Keith; and (iii.) the pressure-pattern theory of Rutherford as elaborated by Ewald.

Resonance Theory.

Although Helmholtz was not the first to suggest that possibly the fibres of the basilar membrane acted as resonators like the strings of a piano, he may be considered the originator of this theory, as he worked out a connected theory of hearing based on the physics of sympathetic resonation.

In his own words, " Suppose we were able to connect every string of a piano with a nerve fibre in such a way that this fibre would be excited and experience a sensation every time the string vibrated. Then every musical tone which impinged on the instrument would excite in the ear, as we know to be really the case, a series of sensations corresponding to the pendular vibrations into which the original motion of the air had to be resolved. By this means, then, the existence of each tone would be exactly so perceived, as it is really perceived by the ear. The sensations excited by the

higher particles under the supposed conditions, would fall to the lot of different nerve fibres, and hence be produced perfectly, separately and independently. Now, as a matter of fact, later microscopic discoveries respecting the internal construction of the ear, lead to the hypothesis that arrangements exist in the ear similar to those which we have imagined. The end of every fibre of the auditory nerve is connected with small elastic parts which we cannot but assume to be set in sympathetic vibration of the waves of sound."

He considered that the radial fibres of the basilar membrane constituted this system of resonators. This membrane increases its width (about three times) as it passes from its beginning in the base of the *cochlea* to its termination in the apex and contains somewhere about 10,000 of these fibres within its substance (Fig. 66).

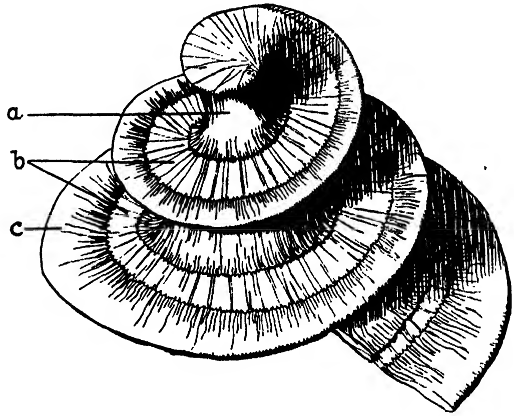


FIG. 66.—Interior of right cochlea (human) exposed to show (*a*) modiolus, (*b*) basilar membrane, and (*c*) lamina spiralis secondarius. Between (*a*) and (*b*) is the lamina spiralis. (E. M. Paul.)

To quote again from Helmholtz, "If the tension in the direction of its length is infinitesimally small in comparison with the tension in the direction of its breadth, then the radial fibres of the basilar membrane may be approximately regarded as forming a system of stretched strings, the membranous connection only serving to give a fulcrum to the pressure of the fluid against these strings. In that case the law of their motion would be the same as if every individual string moved independently of all the others, and obeyed by itself the influence of the periodically alternating pressure of the fluid of the labyrinth contained in the vestibule gallery. Consequently, any exciting tone would set that part of the membrane into sympathetic vibration, for which the proper tone of one of its radial fibres that were stretched and loaded with the various appendages already described corresponds most nearly with the exciting tone; and thence the vibrations will extend with rapidly diminishing strength on to the adjacent parts of the membrane."

That is, three factors are implicated, viz. length, tension and

mass, by variations in which a range of 16 to 20,000 complete vibrations per second could be detected.

Length. It is clear from Fig. 66 that the fibres progressively increase in length from base to apex. Various estimates have been made of the length of the fibres and values ranging from 0·041—0·495 mm. (Hensen) to 0·176—0·4 mm. (Keith), *i.e.* according to Hensen there is a twelve-fold increase in size, while Keith and other modern workers give only a fourfold variation. If the fibres varied in length alone, a thousandfold variation would be necessary to provide resonators tuned to the highest and the lowest note perceptible.

Tension. Reference to the figure (66) will show the presence in the spiral ligament of radiating fibres attached at one end to the outer wall of the cochlear galleries, and at the other end running in a direction continuous with the basilar fibres to which they are attached. At the basal end the ligament is about 0·5 mm. thick, and is full of closely-packed fibres, while at the apical turn it is only about 0·1 mm. thick and contains only a few fibres. Gray and Wilkinson consider that the function of these fibres is to exert a progressively increasing tension on the basilar fibres—applying the greatest tension where the fibres are shortest and thus raising the pitch of the resonators responding to the high notes, and least on the longer fibres, *i.e.* making them capable of resonating to notes lower than they would accept if stretched. (Cf. vibration of stretched string.)

Mass. Most investigators consider that the progressive variation in the length and in the tension of the fibres of the basilar membrane is sufficient to account for the $10\frac{1}{2}$ octaves of the audible range, but Helmholtz vaguely suggested a third physical factor, the load on the fibres. The load is made up of two components, viz. (i.) the organ of Corti and appendages, and (ii.) the endolymph or fluid load. The main reason for postulating load as well as tension and length is to explain why we can hear very low notes, *e.g.* 15–30 vibrations per second. The variation in tension seems adequate for the higher frequencies, but when we come to the lower ones we would need to have very slack strings as resonators on account of their shortness. (i.) The weight of the arches of Corti, the hair cells, sustentacular cells, etc., loads the fibres of the basilar membrane, and so increases their period of vibration. (ii.) Wilkinson considers the fluid load as of fundamental importance. He has constructed models to demonstrate his case, and, if we can accept models as evidence, then he has gone far to prove the correctness of his assumption. The essential feature of his modification of the resonance theory is that the fluid filling the

cochlea acts as a progressively graduated load on the basilar fibres. "Supposing a small transverse sector of the basilar membrane to move from its central position towards the scala tympani, its movement will displace a certain amount of fluid, and the displacement will travel along the scala till the membrane closing the round window is bulged to an extent sufficient to accommodate the amount of fluid displaced. It follows that an equivalent quantity of fluid will be displaced across each cross-section of the scala from the level of the vibrating sector to that of the round window. The mass of the fluid moved will equal that of a column the base of which is equal to the area of the vibrating sector, and the height to the distance from the sector to the round window. A similar mass of fluid will be displaced in the scala vestibuli, but in the opposite direction" (Wilkinson). The mass (weight of double column) progressively increases from the base to the apex of the cochlea. This will cause a differentiation in the periodicity of the vibrations in the same sense as the variations of length and tension, *i.e. length* and *mass* are greatest on the longer fibres, while *tension* is greatest on the shorter fibres.

Now if we consider the mean length of the basilar membrane as 35 mm., we may state as an approximation that the shortest distance (following round the inner curve of the scalae) between the longest fibre of the membrane and the windows would be $2 \times 20 = 40$ mm. The distance between the windows and the shortest fibre is about 2 mm., *i.e.* a twenty-fold variation in fluid load is possible.

Damping. Some mechanism must exist to prevent a resonating fibre from continuing its vibrations after the stimulating sound has ceased. Hartridge has devised many demonstrations of the presence of dampers. In one experiment he used a siren arranged to produce instantaneously a change of phase of half a wave-length in the successive pulses emitted by it. When the change of phase occurred, there was apparently a period of silence. That is, at that moment, the resonators were damped before a fresh set of resonators took up the new vibrations.

This theory receives confirmation from the following facts:—

(1) Birds and other animals whose calls have a short range of pitch have short basilar membranes.

(2) Prolonged subjection to a definite note produces degeneration in a definite part of the membrane—*e.g.* in boiler-makers' disease there is inability to hear high notes with degeneration of the short fibres; animals give evidence of deafness to low notes when the long fibres of the membrane have been destroyed.

(3) If one ear be fatigued by prolonged stimulation from a

constant note, its response to the same note is found to be inhibited. but notes of slightly higher or lower pitch are readily heard.

Against this theory are arrayed most psychologists and not a few physiologists and anatomists. They find in it no adequate explanation of certain phenomena, *e.g.*:

(1) The parrot has only half a whorl of *cochlea,* but is able to imitate speech and to whistle musical notes over a fair range, while the guinea-pig, with one and a half whorls more than man, produces only squeaks and grunts.

(2) The bird has a short basilar membrane, about one-fifth that of the mammal, but has numerous hair cells. These cells are set in the bird in rows of thirty or forty, and in the mammal in rows of four. If equal lengths of membrane vibrate sympathetically to the same note, then as the bird has ten times the number of hair cells stimulated as the mammal, it ought to hear the sound correspondingly louder. This does not appear to be so.

(3) The fibres of the basilar membrane are not separate structures, but are bound together so that we have to deal with a triangular sheet of membrane with the tension applied across the shorter distance. Such a sheet, as we shall see when we consider the pressure-pattern theory, does vibrate as a differential resonator. As a matter of fact, Helmholtz met this difficulty by calculating that a uniform membrane in which the tension was greater from side to side than longitudinally would answer in the same way as a series of fibres as his theory required.

Displacement Theory.

The latest form of this theory is put forward by Sir Thomas Wrightson, an engineer. He is supported by Sir Arthur Keith, the eminent anatomist. He considers that the ear is not a physiological piano played upon by the sound waves, but a delicate spring weighing every phase of a sound wave, simple or compound, and transmitting to the brain a record of every fluctuation of pressure in the endolymph of the *scala media.* Every variation of pressure transmitted by the *stapes* to the perilymph is in turn transmitted to the membrane closing the *fenestra rotunda.* The cochlear system is a closed one, in shape rather like a long-drawn-out, doubled-over hour glass, with the *stapes* operating at one end. The only relief for the motion of displacement is at the *fenestra rotunda,* at the opposite end, whose membrane moves to and fro simultaneously with the *stapes.* These movements are transmitted to the endolymph enclosed in the tube, the *scala media,* which ends blindly at the

helicotrema. He believes that the hair cells and not the basilar membrane are the sensitive organ. They pass through the meshes of the reticulate membrane, and, according to Wrightson and Keith, have their upper ends fixed in the tectoria. The tectorial membrane, it will be remembered, is attached to the spiral lamina, as a nail grows out of the finger. Now, displacement of the fluid causes movement of the whole reticulate membrane. This latter produces a to-and-fro movement of the base of all the hairs, and, as these hairs are fixed in the tectorial membrane, they will bend. " It would only be the simple pure tones which would give to the hairlet a pure symmetrical harmonic motion, but by the displacement of liquid under pressure, every conceivable succession of bendings of the innumerable hairlets can be obtained to convey to the auditory nerve every impulse required to produce the pitch of each resultant and component tone " (Wrightson).

Thus the cubic displacement of fluid is converted, by means of the arch of Corti, into a linear movement of the reticulate membrane, etc. By means of the resistance of the *tectoria*, the linear movement is converted into the bending of the hairlets.

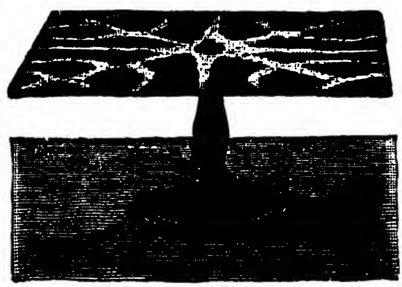


FIG. 67.—Chladni's plate.

Such an explanation fails to account for the ability of the trained musical ear to perceive as separate entities the different sounds from an orchestra reaching it simultaneously, and it does not give a very clear explanation of " tone-gaps," nor does it explain why fatigue to one note leaves the response to all other notes apparently unaffected in intensity.

Pressure-Pattern Theory.

This theory, originated by Waller and elaborated by Ewald, is based on the sand and powder patterns produced on vibrating plates (Fig. 67) (see Chladni's plates, Chap. XVII.). The pattern traced by the sand depends on three main factors : (1) the part of the plate where the vibrations start, (2) the parts of the plate that are fixed or damped, and (3) the frequency of the induced vibrations. Ewald showed that the membrane in his " acoustic camera " vibrated like Chladni's plates, and that the distance between the nodes varied with the pitch of the note entering the camera. The sand comes to rest on the nodal lines, or lines of rest, and makes a pattern, so translating a sound figure into a light

figure. A light powder like lycopodium, on the other hand, is caught up in the air whirling above the moving parts of the plate and comes to rest in heaps where the greatest movements of the plate have been.

Ewald used a rubber membrane stretched over a frame and immersed in fluid. Instead of producing figures in sand, he smeared the surface of the rubber with oil and examined it under oblique illumination by means of a very low-power microscope set at an angle. The acoustic images so produced can readily be photographed, and are found to be characteristic for each note sounded near the apparatus. These images produced on the basilar membrane cause some hair cells to move and so transmit a stimulus to the fibres of the cochlear nerve with which these cells are richly provided. (According to some investigators, the moving part is the *membrana tectoria*, which by coming against the hairs of the cells excites the nerve fibres.)

This theory and that of Helmholtz have this in common, that the primary analysis of the sound takes place in the ear. This is as one would expect from a study of all other external receptors, and, as is the case with the other receptors, the ultimate analysis must take place in the cortex. This final analytical act is not an inborn process, but is acquired through experience.

To conclude in the words of Helmholtz: "On reviewing the whole arrangement there can be no doubt that Corti's organ is an apparatus for receiving the vibrations of the basilar membrane and for vibrating of itself, but our present knowledge is not sufficient to determine with accuracy the manner in which these vibrations take place."

Intensity. Sounds differ not only in pitch, but in loudness and in quality. We must go to Adrian's experiments to find out how the ear differentiates between sounds differing in intensity. It has been found that the *frequency* with which the electric impulses pass towards the sensory centre when a sensory nerve fibre is stimulated, varies with the intensity of the stimulation. That is, a loud sound of a certain pitch would cause a rapid succession of impulses to pass along certain fibres of the cochlear nerve, while less intense sound of the same pitch would produce probably only a single volley of impulses. Now although these sounds were produced, say, by the same tuning fork, vigorously banged or gently touched, and, therefore, of the same pitch, the louder sound would appear to us to be of higher pitch than the less intense sound. This is due to the more intense sound producing some vibration in the hair-cells lying nearer the entrance of the disturbance into the cochlea, *i.e.* nearer the base where the fibres are

shorter. This may be shown clearly on a model ear built to demonstrate either Helmholtz's or Ewald's theory.

Timbre or Quality. Most sound-producing instruments impart a characteristic quality to the sounds emitted by them. Anyone, for instance, could tell whether a specific note were produced from a piano, a violin, a pipe-organ, a harmonium, etc. This is due to the fact that not only does the instrument vibrate as a whole, but each individual part tends to vibrate in a characteristic way. These partial vibrations give rise to overtones, which impart quality to the production in accordance with their number, their pitch and their relative intensities. The ear, therefore, has no need to develop a special mechanism for the purpose of appreciating *timbre*.

We must now consider the physical reasons for the peculiar shape of the cochlea. Why should it take two and a half turns up to the helicotrema, and why should it be a double body, *e.g. scala media* sandwiched in between the *scalae vestibuli* and *tympani?* If the cochlear tube were straight, having at one end the oval window with the stapes and at the other the round window with its compensating membrane, the floor of the tube being the basilar membrane with appendages, and the tube being practically of uniform width throughout, we would have a receptor capable of the maximum distortion possible, and also liable to have induced in its fluid ripples and changes of pressure due to physical changes other than sound waves. Every time that we started to move our heads in space with or relatively to our bodies we would cause a movement of the cochlear fluid and would receive the sensation of sound. The same thing would happen every time we changed either our rate of movement or its direction. What a deafening crash there would be when we violently applied negative acceleration, *e.g.* express train stopping suddenly. This disadvantage could be overcome partially by bending the tube into a U with the two windows close together. A stricture at the bend of the U would further help matters. We would now be able to move linearly in any direction and alter our rate of progression without hearing about it. An angular displacement would still disturb hearing, especially if the movement were at all rapidly initiated or stopped. The winding of the tube into a narrow spiral gives us an organ of hearing containing a liquid that undergoes very little relative displacement with the ordinary movements of the body. It still tends to generate sounds when extraordinary motion or acceleration is applied to the body.

The double nature of the organ is a structural necessity. The basilar membrane must vibrate in some fluid (gas or liquid)

medium. As fluid fills the space on one side of the membrane, efficiency demands a fluid on the other side. One may also say that the separation of the scala media from the scala vestibuli enables (i.) damping to take place readily, and (ii.) a simpler and more efficient balance-organ (labyrinth) to be constructed.

Binaural Hearing.

Quite apart from the undoubted fact that having two ears enables us to hear more distinctly and allows of the damage of one ear without completely shutting us out from the variations in external sound, the possession of two listening points alone permits of the *localisation* of sound. The head is oriented so that an equal intensity of sound falls on each ear, *i.e.* if our ears are equally sensitive, we face the sound—we are positively audiotropic (Chap. XXXIII.).

There remains one very important matter which should be considered because of its diagnostic value to the physician, viz. conduction of sound waves by the bones of the head. It is common knowledge that sound vibrations travel more readily through a solid than through a liquid or a gaseous medium. A watch, placed sufficiently far away to be inaudible, can be heard ticking if touched by a lath held between the teeth. If something goes wrong with the mechanism of the ear, one wants in the first place to locate the fault. Is the external ear, the middle ear or the internal ear the seat of the trouble ? The test is usually made by placing a vibrating body, such as a tuning fork, on one of the cranial bones. If the sound is not appreciated, then the fault lies within the internal ear. Either the organ of Corti (or its nervous attachments) have broken down or the membrane of the *fenestra rotunda* is not normal. Provided the organ of Corti and its nervous attachments are intact and the round membrane is flaccid, sounds may be heard by bone conduction, and ordinary hearing is not impossible. The vibrations are transmitted directly through the thick, exceedingly dense but elastic bony walls of the aural cavity, and produce a movement of the basilar membrane, etc. This can only take place if the membrane of the round window is functioning properly, or if the *stapes* moves normally in its oval window. If the openings were to lose their elastic windows and not move to and fro with every condensation and rarefaction, then the cochlea would be, to all intents, a sealed cavity filled with fluid. Such fluid could not oscillate ; it could be alternately compressed and released from this extra pressure, but this slight molecular movement could not stimulate the hairlets.

Further, if both the *stapes* and round membrane were free to move, hearing in the case of a diseased middle ear would not be so good as when only one of the pair were free. This is because part of the displacement of the cochlear fluid caused by the vibrations of the surrounding bone is dissipated by moving the *stapes* outwards. People may hear fairly well after the *stapes* has become immovably fixed in the *fenestra ovalis*. In cases where the drum of the ear has been punctured, hearing may be improved by fixation of the *stapes*, *e.g.* by application of a plug of cotton wool.

When sounds are conducted to the inner ear by means of the bones of the skull, in people with normal hearing, the intensity of the sound is markedly increased if the movement of the *stapes* is hindered. For example, if a vibrating tuning-fork is placed on the region of the interparietal suture, when both ears are unobstructed and normal, sound is heard equally by both. If the drum of one ear and appended ossicles are hindered from taking a full excursion by blocking the *meatus* with a finger, the sound appears most distinctly at this ear. When both ears are treated in this way, localisation is again median. A common entotic phenomenon is the audibility of the pulse in an obstructed ear. It may be due to the transmission of the pulse-wave oscillation to the air of the middle ear—which acts as a resonator—reinforcing the vibrations and then transmitting them to the internal ear. It is more probable, however, that the beat of the carotid artery is transmitted through the parietal bone direct to the fluid of the cochlea.

Balance.

The ear is a double organ. We have just dealt with its function as the receiver and analyser of certain vibrations in matter transmitted to it generally through the air, but capable of transmission through much denser media. The other and more ancient function is that of giving information as to (i.) the position of our head in space, and (ii.) the acceleration of the head in space.

Structure (Fig. 63). The vestibule (Fig. 63 (9)) and the semicircular canals (10), like the cochlea (6), are double organs. The outer or osseous part is hollowed out of the substance of the bone, lined by periosteum and filled with perilymph in which the membranous part is placed. The various parts of the membranous labyrinth, viz. (*a*) *utricle* and *saccule*, contained in the vestibule, (*b*) three *semicircular ducts*, with their ampullae, in the osseous semicircular canals, and (*c*) the *scala media* in the cochlea are filled with endolymph and are in fluid connection with one another. The semicircular ducts open into the utricle, the utricle into the

saccule through the *ductus endolymphaticus*, and the saccule into the scala media through the *canalis reuniens*. Nerve fibres from the vestibular division of the auditory nerve end in naked fibrils between the hair-cells of the maculae of the utricle and saccule and of the cristae ampullares of the semicircular ducts. The maculae and cristae are little thickenings on the internal surfaces of these cavities, one in each. The epithelium on the surface of the humps is columnar, and consists of (i.) fusiform supporting cells, the free ends of which unite to form a thin cuticle, and (ii.) flask-shaped hair-cells, whose free ends are surmounted by a long, tapering, hair-like filament. Two small rounded calcareous bodies termed otoconia, or otoliths, lie in contact with the free ends of the projecting hairs of the maculae.

Mechanism of Utricles and Saccules.

It is obvious that any movement of the hairs will cause a

FIG. 68.—Rabbit's skull with oriented magnified models of the utricle and saccule maculae. Skull in normal position. The surface of the macula to which the otolith is attached is indicated in the utricle by white dots and in the saccula by white stripes on the black plate. Stereoscopic. (Magnus, "Körperstellung.")

stimulation of the filaments of the vestibular nerve, and that the otoliths are an excellent means of providing this stimulation. But the exact way in which this occurs does not appear to be so simple a matter to determine. The literature on this question contains somewhere about 2,000 papers, and they do not all convey the same impression. To begin with, most of the work has been done on fishes, where operative procedure is simpler than in the mammal. One cannot directly apply knowledge so gained to the human being, because the structure and function of the organs are somewhat different. The present view, largely due to experiments on the rabbit and other mammals by Magnus, is that an alteration of the position of the head brought about by allowing gravity to act on the otoliths causes them to exert a slight pull on the hairs, and so to induce the passage of a nervous impulse which in turn affects the muscular tone of the body.

Utricles. The utricle in each ear has one macula with its otolith.

The maculae are so placed that when the head (of the rabbit) is held in its normal position they lie with their hairs vertical, bearing the otoliths above them. Now, if the rabbit is held so that the head has turned through 180 degrees and the macular hairs are again vertical, but the otoliths are now hanging from them, it has been found that the limbs are extended maximally. That is, when the otolith in *each* macula *presses* against the hairs, one has minimal limb tonus, and, conversely, when the otolith in *each* macula *pulls* on the hairs, one has maximal tonus. Degrees of tonus may be obtained by degrees of rotation.

Saccules. The saccule of each ear has its macula on the inner lateral wall, *i.e.* in the normal position of the head (of the rabbit) the hairs will be horizontal and pointing in opposite directions in each ear. The macula of the saccule is least stimulated when the otolith *presses* against the hairs, and undergoes maximal stimulation when the otolith *hangs* vertically from the hairs (cf. utricle). That is, *e.g.* when the head is hanging over towards the left side of the animal, the right saccule will receive minimal and the left saccule maximal stimulation, producing an asymmetric alteration of muscle tonus, the extensor muscles of the limbs on the left side having their tone increased and those on the right side undergoing diminution of extensor tone, whereby a "righting" action is developed (Fig. 68).

Semicircular Canals. These structures, of which there are six, are arranged so that the three on each side are in three planes at right angles to one another. The two canals, which lie externally in each ear, are situated in a plane which is almost horizontal in the erect position of the human head. The two other pairs of canals are, therefore, vertical, as they are at right angles to the external canals. The two groups of three canals each are set, as it were, back to back, *i.e.* mirror images of one another, so that any rotatory movement of the head will tend to produce equal and opposite movements of the fluid pressure in the canals and their membranous ducts. The ducts running within the osseous canals occupy about one-fourth of the volume of the canals except where they are widened to fill the ampullae, which are bulbous cavities at one end of each canal (anterior end of external canals and external ends of anterior and posterior canals). The bony ampullae are about twice the diameter of the canals, and, therefore, each of the membranous ducts undergoes an 800 per cent. enlargement. On the concave side of each membranous ampulla is a thickened ridge rising almost to the axis of the duct and covered with columnar cells forming the crista ampullaris. From the surface of the crista project long flexible hairs, thicker and more bristle-like

than ordinary ciliated epithelium (*q.v.*), and held together by a mucous gelatinous mass so that they cannot move freely in the endolymph. The hair-cells are supplied with fine filaments from the vestibular division of the eighth nerve.

The ducts on each side open into the corresponding utricle by five orifices, *i.e.* two of the ducts (the anterior and the posterior) join in a common canal (*crus commune*) and have a common opening into the utricle.

Mechanism. The structure of the organ indicates that alterations of the pressure of the viscous fluid in the membranous ampullae will tend to bend the hairs and, following the same scheme as in the maculae, will alter muscle tone. When a rotatory movement round any axis is initiated or accelerated positively or negatively, positive or negative fluid pressure beyond the normal will tend to develop in certain ampullae and so produce stimulation of the cristae. Initiation of rotation, for instance, of an animal about its dorso-ventral axis, *i.e.* in the plane of the horizontal canals, produces the same results as mechanical stimulation of the hairs with a tiny pledget of cotton wool. Adaptation occurs gradually on continuing the rotation at a steady rate. That is, like the proprioceptors already studied, adaptation is a slow process. Any alteration of rate, quickening, slowing or stopping acts as a fresh stimulus.

It has been shown conclusively by Mach that actual currents cannot be produced either in the perilymph of the canals or in the endolymph of the ducts. Maxwell has disposed of the theory that fluid pressure is developed in the duct and transmitted to the ampulla. He tied off the *posterior* end of the *external* canal (horizontal) and, after cutting it, raised it so that its plane was vertical. He then rotated the animal about its dorso-ventral axis and produced the usual reactions. That is, the animal reacted normally when it was possible only for fluid to enter the ampulla from the utricle. *The normal mechanism, therefore, for excitation of the nerve-endings in the cristae is the transmission of fluid-pressure from the utricle to the ampullae.* This pressure is prevented from rapid dissipation by the narrowing of the duct by eight times as it leaves the ampulla.

The otoliths of the maculae and the hairs of the cristae are both capable of responding to angular and linear acceleration. Maxwell has shown that the otoliths, because of their greater specific gravity, have a greater inertia than the fluid they displace and, therefore, are as likely to suffer displacement during rotation as the endolymph. There is also a physical possibility that during linear and angular accelerations the utricular fluid would tend to lag and

heap up pressure in some ampulla. Some work on fishes by Maxwell and others gives an indication that this co-operation is extremely probable.

The alterations in extensor tone produced by stimulatino of the hair-cells of the vestibular apparatus are augmented by stimuli coming from the muscles themselves, especially from those of the neck, and by auditory and visual stimulation. The following sentences taken from "Recent Advances in Physiology" may make this clear. "Suppose that a cat hears a mouse moving on its right. The head is turned to the right; this alters the centre of gravity, but as a result of the tonic neck reflex, there is an increase of tonus in the muscles on the right side of the body, which preserves the balance by throwing the weight on the right limbs. Now, because the left limbs are less loaded, they will be the first to move if the animal springs to its prey, so that the cat will automatically move in the right direction." Compare with this statement the description given in Chap. XXXIII. of Hammond's heliotropic dog.

Further Reading

Hartridge. In Starling's "Principles of Physiology." J. & A. Churchill.
Lovatt Evans. "Recent Advances in Physiology." J. & A. Churchill.

CHAPTER XXI

OUTPOSTS OF THE INTELLIGENCE SERVICE

(c) DISTANCE RECEPTOR FOR LIGHT

THE EYE

"I had rather not describe it at all, so that neither the difficulty of the explanation nor its length might cause me to be hated." GALEN.

THE following points in connection with light are recalled to the student's memory.

(1) It can be shown in various ways that light travels in straight lines, *e.g.* interception of light by opaque objects.

(2) The path along which light travels from each point of a luminous object is a *ray*. A collection of such rays converging so as to form a cone is termed a **pencil of rays.** When the apex of the cone is at the source of light the *pencil* is *divergent* ; when the reverse is the case, one has a *convergent* pencil. When a pencil of rays diverges from or converges to a point 6 metres or more away (infinite distance) the rays are considered to be *parallel.*

(3) The simplest instrument with which to obtain an image of an external object is a **pinhole camera,** which consists of a rectangular box. One end of the camera is made of ground glass, and in the centre of the opposite side is a pinhole. Rays of light diverge from a luminous object, say an arrow (A—W) placed in front of the pinhole (O). A divergent pencil of rays proceeds from the arrowhead A. A certain very small collection of rays from A will pass through O, and reach the screen S. The result will be an image A' of A on the ground glass. Similarly, every other point, RRO and W, of the arrow will produce divergent pencils of rays, some of which rays will pass through O to give a complete image, $A'R'R'O'W'$. Now as all the rays entering the camera have passed through the pinhole, it follows that they must have crossed at O, and, therefore, the image will be inverted, viz., $W' \ldots A'$. There is a definite relationship between the relative sizes of object and image and the relative distances of pinhole to screen OS and pinhole to object OR, *i.e.*:

$$\frac{\text{size of image}}{\text{size of object}} = \frac{\text{distance } OS}{\text{distance } OR}$$

By varying the position of the screen, the image may be made to vary in size, and similarly, but in the reverse way, the size of the image depends on the distance of the object from the camera. By increasing OS or reducing OR within certain limits, the image may be made larger. When OR is reduced to a certain size, the pencils of rays coming from the points A and R, O and W miss the pinhole with the exception of the most divergent rays. The image is, therefore, blurred. The further the object is from the camera (within limits) the more will the less divergent rays from the extremities of the

arrow have a chance to pass into the camera, producing a well-defined image. That is, the pencils from every point of the image consist of parallel rays.

In the pinhole camera the rays of light traverse an *optical medium* which is *homogeneous*, *i.e.* it is identical in its properties at all parts. The rays would take a somewhat different path if the optical medium were heterogeneous. For example, the medium might be denser or rarer at one part. When the ray came to pass through this part it would suffer refraction.

(4) **Refraction.**—When a ray of light passes from a rare to a dense medium (or *vice versâ*) it undergoes refraction, *i.e.* it is bent towards (or away from) the perpendicular to the surface at the point of incidence. This perpendicular is called the normal. Snell's Law states that for any two media, the sine of the angle of incidence bears a constant ratio to the sine of the angle of refraction.

Refractive Index. In Fig. 69 PO is the incident ray, OQ the refracted ray,

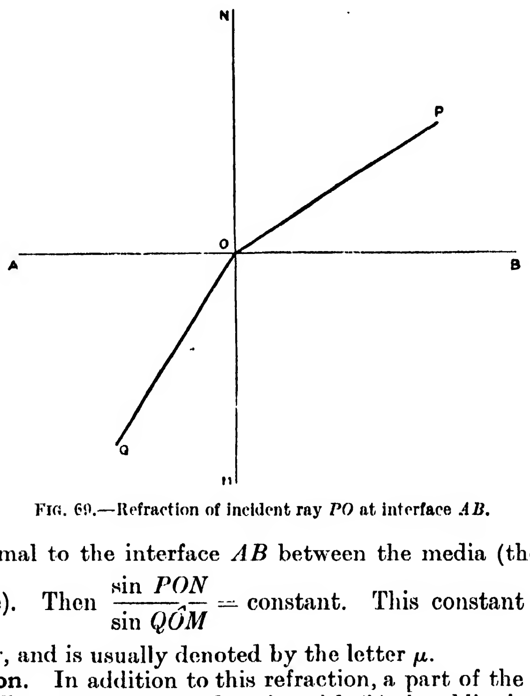


FIG. 69.—Refraction of incident ray PO at interface AB.

NOM the normal to the interface AB between the media (the upper being the less dense). Then $\dfrac{\sin PON}{\sin Q\hat{O}M} = \text{constant}$. This constant is called the *refractive index*, and is usually denoted by the letter μ.

(5) **Reflection.** In addition to this refraction, a part of the incident light is reflected. The amount reflected varies with (i.) the obliquity of incidence, (ii.) the difference in refractive index.

(6) **Lenses.** A lens is a portion of any transparent medium bounded by surfaces that are parts of a spherical surface. The line joining the centres of the two spheres which bound a lens is called **the principal axis of the lens.** There are two main types of lenses, viz. convex and concave. The former cause rays to converge to a point on the principal axis, whereas the concave lens causes rays apparently to diverge from a point on the principal axis. The point to which or from which the rays appear to converge is, *in the case of parallel rays*, the **principal focus** of the lens.

Focal Length. The distance between the principal focus and the lens is

(for a thin lens) its focal length. If the focal lengths are given in metres, then their reciprocals give the power of the lens in dioptres. For example, a lens with a focal length of 1 metre is of 1 dioptre; of 2 metres of 0·5 dioptre; of 0·3 metres of 3 dioptres, and so on. Convex lenses are *positive*, and their power in dioptres is given with the sign +. The negative sign is placed with the dioptric value of concave lenses.

When a source of light, *e.g.* a candle, is placed near a biconvex lens, we see very clearly two images produced by reflection. The first is formed by the anterior convex surface and is upright, the second is formed at the posterior surface, which is *concave* in respect of rays passing out of the lens into the atmosphere, and is inverted. The *size* of the image decreases as the convexity of the lens increases. Its *brightness* increases the more obliquely the rays from the candle strike the surface and also with increase of the refractive index of the medium composing the lens.

If we now replace the pinhole of the camera with a convex lens, we will find that we still obtain an image of the arrow on the screen. The introduction of the lens brings advantages and disadvantages. Two of the chief drawbacks are chromatic and spherical aberration.

Chromatic Aberration. When white light, which is a mixture of waves of various frequencies (*q.v.*), passes through a lens, each monochromatic con-

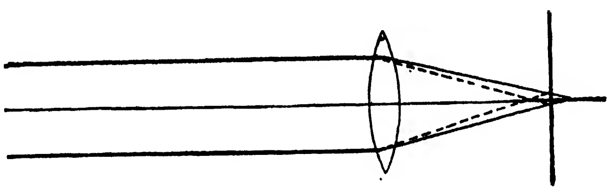


FIG. 70.—Chromatic Aberration.

stituent is refracted to a degree depending on its frequency. That is, the refractive index of the lens has a different value for each type of light, and therefore the different waves, when they strike the bounding surfaces of the lens, will undergo different deviations. Those of high frequency, violet rays, are refracted to a greater degree than the slower, longer red rays.

In Fig. 70 the dotted lines represent the path taken by limiting parallel violet rays, while the continuous lines coming to a focus further away from the lens represent the paths of the red constituent rays of white light. If the screen is placed as in the diagram midway between the principal foci for violet and red rays, the image of the arrow will appear surrounded by a red and violet halo.

Chromatic Difference of Magnification. This defect also is due to the unequal refraction of waves of different frequencies. Not only do the foci of the various monochromatic components of white light fall on different parts of the principal axis, but their pencils form different angles with the optic axis. Thus, rays entering the lens at a considerable angle come to a focus at a point depending on the colour of the light and therefore the size of the image produced will also depend on the colour of the light. That is, violet rays will produce an image smaller than that produced by blue rays, blue smaller than green, green smaller than yellow, while the largest images will come from red rays.

These defects may be overcome by the use of a combination of lenses,

convex and concave, made of substances of different refractive index, or by the insertion of colour filters to cut out a series of frequencies.

Spherical Aberration. Rays of light passing through the peripheral part of a lens are refracted more than those passing through the central part. This tends to produce distortion of the image at the periphery.

Curvature of Field is found in all convergent lenses of simple formula. For example, the lines on a piece of squared paper examined through a positive lens of about 1 or 2 inches focus will appear definitely curved, all except the two lines at right angles to one another occupying the centre of the field of vision. Photographs taken with the iris diaphragm wide open, if only a single lens is used, will exhibit this aberration to a marked degree, *e.g.* sides of buildings, etc., will appear curved, each part of the picture will be confused, being formed by pencils refracted through various parts of the lens.

Spherical aberrations may be avoided by the employment of a lens whose curvature gradually decreases from centre to periphery, and by placing an iris diaphragm or stop in front of the lens to cut off peripherally incident rays.

For a lens (of crown glass) to produce absolutely the smallest possible amount of aberration it should be biconvex, the radii of curvature of its surfaces being in the ratio of 1 : 6, *the more strongly curved surface facing the incident rays.* Such a lens is termed a **crossed lens.** Crown glass has a refractive index of 1·5. If a glass with a $\mu = 1{\cdot}6$ (flint glass) were used the side away from the incident light would be flat, *i.e.* the lens would be plano-convex.

Comma. Rays coming from a point source form, with some lenses, an image with a fine tail pointing towards the optical axis—just like an illuminated comma. This aberration is seen easily with the old-fashioned carafe, which when filled with water acted as a lens. The distortion is due to a difference in the position of the image produced by different zones of the lens. If the lens obeys the sine law it is free from comma.

The camera, whether of the pinhole variety or fitted with a good lens and diaphragm, may produce images which suffer from defects not due to the optical system, *e.g.* halation, flare, irradiation and scattered light.

Halation is noticeable only when the photographic plate or screen is sufficiently thick for the image formed on the surface to be reinforced by a reflection of the image from the internal glass surface, so producing a blurred outline or halo. Photographic films, owing to their thinness, do not exhibit halation noticeably.

Flare is due to the illumination of the screen by light reflected from the internal surfaces of the compound lens. It is least when the refractive indices of the heterogeneous optical medium are almost identical and when the light passes through the lens along the optic axis. The greater the angle of incidence of the rays and the greater the differences in refractive index of the components of the optical medium, the greater will be the possibility of flare.

Irradiation is the spreading of the image on the screen due to excess illumination of the object. It is easily produced in photography, *e.g.* by pointing the camera directly to the sun and getting apparently a "moonlight scene."

Scattered light is light reflected from the inner surface of the camera, *e.g.* shiny bellows, badly blacked walls, etc.

We will deal later with the ways in which the eye avoids these defects, meanwhile we will consider the structure of the organ.

Anatomy of Eye.

To understand the mechanism of the eye even from the purely physical standpoint, *i.e.* as an optical instrument, it is necessary to have a clear conception of its structure.

Anatomy (contributed by J. Secker). The human eyeball (Fig. 71) is a hollow sphere of about 20 mm. in diameter. It consists of three concentrically arranged coats enclosing a cavity containing three refracting media. These coats are (i.) an outer fibrous envelope which is divided into an opaque portion, the *sclera*, and a transparent portion, the *cornea*. The sclera constitutes the posterior five-sixths of the coat, and the cornea, which has a greater convexity than the sclera, the remaining anterior one-sixth. (ii.) A richly pigmented coat, the *chorioid*, which is the vascular tunic of the eyeball, and contains the intrinsic muscles of the eye, is the intermediate coat. At the junction

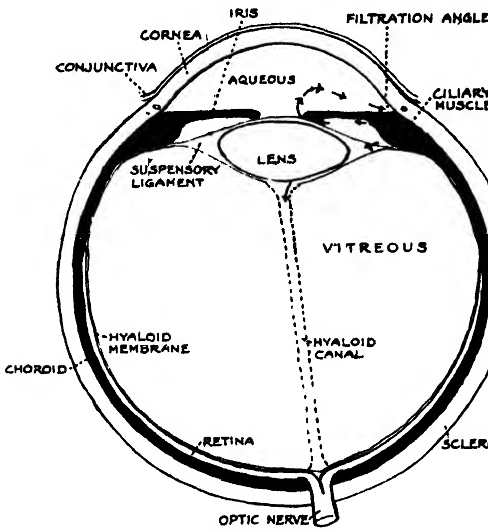


FIG. 71.—Diagrammatic section through equator of the left eye seen from above.

of the sclera with the cornea, the chorioid ceases to be in intimate contact with the sclera and projects as a curtain, the *iris*, into the cavity of the eye, dividing the space between cornea and vitreous humour into an anterior and a posterior chamber. In the centre of this curtain there is a central circular aperture of variable size, *the pupil*. In the substance of the iris are two sets of muscle fibres, one set, the constrictor muscles, arranged concentrically with the pupil, and the other set, the dilator muscles, arranged radially. At a point immediately posterior to the iris a series of about seventy radially arranged processes project into the cavity. These projections or *ciliary processes* consist of connective tissue containing blood vessels, and supplied with muscular fibres from the ciliary muscle, which has its main body in the chorioid coat at the region from which the ciliary processes originate. The ciliary muscle arises from a spur of the sclera at the corneo-sclerotic junction and consists of two sets of fibres, a circular and a radial set. The latter passes into the ciliary processes, and together with them constitutes the *ciliary body*. (iii.) The innermost coat, the retina, is the sensitive layer of the visual

apparatus and corresponds to the plate of a camera. Histologists divide the substance of the retina into eight layers, viz. starting from the side on which the light falls, *i.e.* next to the vitreous humour :

1. Stratum opticum. Layer of non-myelinated nerve fibres.
2. Ganglionic nerve cell layer.
3. Inner molecular layer. Interlacing dendrites of 2 and 4.
4. Inner nuclear layer. Bipolar nerve cells.
5. Outer molecular layer. Dendrites of 4 and 6.
6. Outer nuclear layer. Neurones of rods and cones.
7. Bacillary layer. Layer of rods and cones.
8. Stratum pigmenti.

For our purpose we may consider four sets of elements in the retina, viz. neurones, rods, cones, and pigment containing cells.

(*a*) The bacillary layer contains structures known as rods and cones. These structures are believed to be the actual sensitive structures of the eye. Under certain conditions, *e.g.* when the eye has been in the dark for some time before death, fine processes of the pigment cells can be seen to pass up between the cells of this layer. (*b*) An intermediate layer of bipolar cells which function as connector neurones and link up the rods and cones with the next layer of neurones. (*c*) The ganglion cells.

The axons of the ganglion cells pass horizontally across the inner surface of the retina and converge on a point at the back of the eyeball slightly internal to and just below the antero-posterior axis, to pierce the chorioid and sclera to form the optic nerve. These layers, as mentioned above, do not, however, extend so far anteriorly as the ciliary region, but are represented in this region by a double layer of pigmented cells constituting the *pars ciliaris retinæ*. The sensitive retina itself shows variations in structure in different regions.

At a point where the antero-posterior axis meets the retina there is an area which is yellow in colour, the *macula lutea*, in the centre of which is a depression, the *fovea centralis*. At the fovea, which is the area for direct vision, only cones are found, and here the cones are larger than in other areas of the retina. In addition to the absence of rods at the fovea the remaining layers of the retina are not represented, the centrally directed processes of the cones diverging towards the periphery of the macula to end in relation to the ganglion cells, which are at this region found to be of several layers deep.

The retina receives its own arterial blood supply from the arteria centralis retinæ, a small artery which enters the eyeball at the site of exit of the optic nerve. Branches of this artery radiate on the inner surface of the retina, supplying all areas excepting the fovea centralis.

The three coats as described constitute the walls of the eyeball and enclose the three refractive media, *i.e.* the aqueous humour, the lens and the vitreous body.

The **lens** is a laminated biconvex, transparent, elastic structure, the posterior surface of which is more convex than the anterior surface. The lens is placed just behind the iris and centred with the pupil, and is enclosed in an elastic membrane called the capsule. The periphery of the capsule is attached by a thickened portion of the hyaloid membrane (*vide infra*), known as the *suspensory ligament*, to the ciliary processes.

The anterior compartment of the eyeball contains a clear fluid, the aqueous humour, and the posterior portion a more jelly-like substance, the vitreous body. The vitreous body (or humour) is enclosed in a membrane, the

hyaloid membrane. At the back of the eye, this membrane is in intimate contact with the retina, but on reaching the ciliary region it splits into two layers, the posterior of which is continued over the anterior surface of the vitreous body, and the anterior, gaining attachment to the ciliary processes, becomes thickened to form the suspensory ligament of the lens.

On the anterior surface of the vitreous body is a cavity, the hyaloid fossa, in which the posterior surface of the lens is lodged. From this fossa a minute canal, the hyaloid canal, passes obliquely backwards to the point of exit of the optic nerve.

Recent work on the anatomy of the living eye with the slit-lamp seems to suggest that a small space, the retro-lental space, exists between the posterior surface of the lens and the anterior surface of the vitreous body, and that the aqueous humour is able to pass between the fibres of the suspensory ligament into this space, which is drained by the hyaloid canal into the lymphatics of the sheath of the optic nerve, or into the retinal vessels.

The Eye as an Optical Instrument.

The physics of vision may be considered under two heads, viz. (1) the way in which the image is produced on the retina, and (2) how that image stimulates the end-organs for vision in the retina so that impulses pass to the optic nerve, producing finally a change in consciousness. Under the former head will fall the study of the defects common to a camera and the means by which they are overcome in the eye.

(1) There can be no doubt as to the actual formation of an inverted image on the retina. If a small window be cut through the back of a freshly excised eye, and the space covered with a sheet of tissue paper or a small bit of ground glass inserted, inverted illuminated images of objects placed before the eye may be seen on the screen. The optical system consists of (*a*) cornea, (*b*) lens, and (*c*) iris. Light will undergo refraction at three surfaces, *e.g.* where it enters the cornea and where it enters and leaves the lens. This may be deduced from consideration of the refractive indices of the various media of the system given below.

Refraction also occurs at the posterior surface of the cornea, but as the R.I. of cornea and aqueous humour differ only slightly, we may neglect refraction at this surface.

TABLE XXXVII

REFRACTIVE INDICES OF MEDIA

Air	1·00
Cornea	1·37
Aqueous humour	1·33
Lens (periphery)	1·37
(central nucleus)	1·41
(total equivalent)	1·42
Vitreous humour	1·33

At each of these three surfaces the light passes from a medium of one density to another and, therefore, as the surfaces are convex, the incident ray is bent towards the central axis.

We have seen that refraction is always accompanied by a certain amount of reflection, and this is the cause of the phenomenon known as *Sanson's Images.* If a candle is held at a short distance from one side of the eye, the observer can distinguish the images formed by each of the refracting surfaces. The image produced by the cornea where the change in refractive index is from 1 to 1·37 is much brighter than the images from the lens, where the change is from 1·33 to 1·42 and from 1·42 to 1·33. In the case of the two latter also there is a certain amount of absorption of light by the media. The images from the cornea and the anterior surface of lens are upright, that from the posterior surface of lens is inverted.

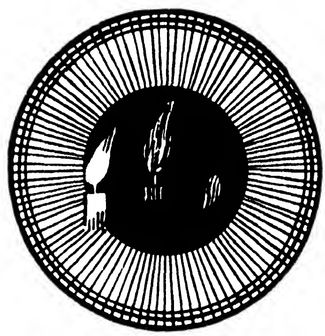


FIG. 72.—Sanson's Images.
The images, from left to right, are from the anterior surface of the cornea, anterior surface of lens, and posterior surface of the lens. (From Goulden's "Refraction.")

Curvature of the Surfaces. The images of the candle are not all of the same size, because the radii of curvature of the three reflecting and refracting surfaces are different. The central image is the largest because the anterior surface of the lens is the least curved, while the inverted image is the smallest because it is reflected from the posterior concave surface of the lens which has the greatest curvature, *i.e.* smallest radius of curvature (Table XXXVIII.).

TABLE XXXVIII

RADII OF CURVATURES OF CORNEA AND LENS

	mm.
Cornea (anterior surface)	7·98
(posterior surface)	6·22
Lens (anterior surface)	10·20
(posterior surface)	6·17

Positions on Optic Axis. If we add to our knowledge of the refractive indices and radii of curvature of the components of the optical system of the eye, measurements of their distances from the retina, we shall be in a position to calculate the dioptric values of these media.

The distances are given in Table XXXIX.

TABLE XXXIX

	mm.
Anterior surface of cornea to aqueous humour	1·15
Anterior surface of cornea to lens	3·54
Anterior surface of cornea to vitreous humour	7·60
Anterior surface of cornea to retina	22·6

The cornea will, therefore, have a focal length of 32 mm., and the lens of 56·3 mm. The dioptric value is, as we have seen, the reciprocal of the focal length, *i.e.* cornea 31 and lens 18 dioptres. It will be seen from these figures that the cornea plays the major part in the formation of the image. When one attempts to see while immersed in water, one finds it impossible clearly to perceive objects near at hand, while more distant objects appear reasonably distinct. Water having a refractive index of a value close to that of the cornea, lengthens the focal distance of the cornea, *i.e.* the eye becomes long-sighted and cannot bring near objects into focus. When the lens is removed for cataract, the cornea has to be strengthened optically by a spectacle lens of about 10 dioptres.

The lens is an interesting structure. It is not homogeneous, but is formed of a series of concentric layers of material graded in optical properties, so that the refractive index increases layer by layer from capsule to nucleus. The curvature of these layers also increases in the same direction, *i.e.* the nucleus has the greatest curvature, appearing almost spherical. This peculiar structure gives the lens increased power. If its composition were uniform, with a mean refractive index of 1·39, its power would be proportional to the difference between its R.I. and the R.I. of the adjacent medium, *i.e.* $1{\cdot}39 - 1{\cdot}34 = 0{\cdot}05$. But its actual R.I. is 1·42. It has thus increased in power in the ratio 8/5.

Focussing.

Every one knows that in a photographic camera it is necessary to adjust the distance between plate and lens in order to focus sharply objects at varying distances. The eye, regarded as an optical instrument, must suffer from this disadvantage, and it is a matter of daily experience with us that near and far objects cannot be seen clearly at the same time. How does the eye overcome this difficulty? The eyeball is rigid and the lens practically fixed. No change in the relative positions of the latter and the retina is possible. The adjustment, called *accommodation*, is brought about by changes in the lens, so that the eyeball has virtually a series of lenses of varying strength, from which it selects the one most suited to the requirements of the moment.

The lens suspended in the resting eye has not its natural shape; it is kept somewhat flattened by the tension of the capsule. If this tension can be relaxed, the lens will become more convex on account of its inherent elasticity. A mechanism for bringing this about is present. The ciliary muscle is fixed at the corneo-sclerotic junction. When its radial fibres contract they drag the ciliary processes, with the adherent hyaloid membrane, forward. Simultaneously the circular fibres by their contraction constrict the circle round which the suspensory ligament is attached. The

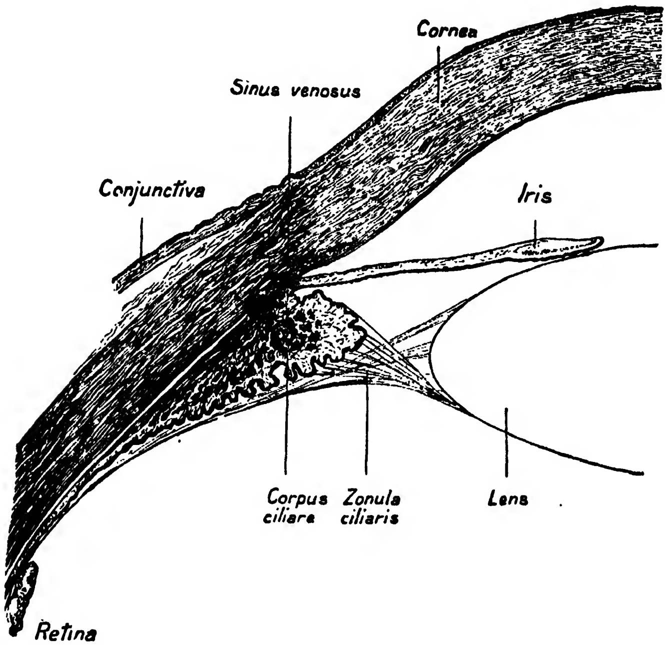


FIG. 73.—Section of anterior parts of eyeball, showing structures concerned in accommodation. (After Merkel and Kallius.)

result is to relax the ligament and lens capsule. and the elasticity of the lens comes into play. The maximum alteration in radial curvature, which affects the anterior surface almost exclusively, is from 10 mm. resting to 6 mm. fully accommodated. The normal adult is thus enabled to see objects distinctly to within 10 or 15 cm. distance.

The Purkinje-Sanson images during the process of accommodation provide a clear demonstration that the main surface affected is the anterior surface of the lens. The central, larger but less bright erect image is reflected from this surface. If, therefore, the two other images remain almost unchanged, and only this image alters in size and position when the eye of the subject, resting on a

distant object, is brought to bear on something near the eye, it is proof that the *main* change of curvature is that of the front of the lens. In accommodation, the central image becomes smaller and moves towards the other erect image. (There are other theories of the mechanism of accommodation, but the above has, at present, the greatest weight of evidence in its favour.) (Fig. 72.)

The iris corresponds to the stop or diaphragm of the camera and is used for the same ends. The way in which the size of the aperture, the pupil, is altered has been considered. It is controlled from the retina, light falling on the retina producing constriction. The function of the iris in accommodation is to increase the depth of focus of the eye. The depth of focus in the case of the eye is the greatest distance through which a luminous point can be moved and still produce a sharp point image (*i.e.* falls on one cone) on the retina. For example, a point may be seen clearly at, say, 100 metres, and at 0·5 metre, *i.e.* the depth of focus, in this case, would be 100 − 0·5 = 99·5 metres. Now in any lens system the depth of focus may be modified by the size of the aperture in front of the lens. The depth increases as the diameter of the aperture decreases. In other words, when we are near an object we use a small stop so that light which might cause the image to spread itself over the retina is shut out, *i.e.* we get definition.

How Optical Defects are Prevented in the Eye.

At the beginning of this chapter we mentioned some of the commoner faults found in lens cameras, *e.g.* spherical and chromatic aberration, comma, halation, flare, irradiation and scattered light, and we discussed methods of overcoming these defects (*q.v.*).

Spherical Aberration. In ordinary circumstances this aberration of the eye is negligible, due (*a*) to the special structure of the lens whereby the rays are refracted in exactly the opposite way from that produced by an ordinary biconvex lens ; that is, the rays passing through the nucleus are more highly refracted than the rays falling on the peripheral parts of the lens, and (*b*) to the stopping action of the iris.

Curvature of the Field. This is avoided completely by the curved screen on which the image is formed. The focal length of the eye (15·5 mm.), being somewhat longer than the radius of curvature of the retina (10 mm.), gives an optical system practically perfect in this respect.

Chromatic Aberration. Experiment shows that the curvature of the anterior surface of the lens and the diameter of the pupil are so adjusted that the image is formed on the retina by the component rays of white light present in greatest intensity. For

sunlight these are the yellow rays (Fig. 1), and other faint blurred images are produced by the violet-blue-green which come to a focus in front of the sensitive surface and by the red rays which would focus behind the retina.

Chromatic Differences of Magnification. The fovea, the area of the retina most sensitive to light (*q.v.*), is eccentric and, therefore, violet, blue and green images should appear smaller on it than yellow and red ones. This manifest fault, however, acts as a corrective to chromatic aberration.

Comma. This is not shown by lens systems obeying the sine law. Apparently the optical system of the eye fulfils this condition.

Halation does not occur in the normal eye, as the reflecting layer and the sensitive layer are practically identical.

Flare. Table XXXVII. shows that the refractive indices of the media bounding the lens differ only slightly from that of the lens itself. Further, the R.I. of the peripheral parts of the lens is even closer to the R.I. of the humours. Flare is therefore negligible.

Irradiation. This does occur in the eye if the illumination is very bright, and causes bad definition of objects.

Scattered Light. In spite of the liberal supply of protective pigment in the layer of the retina lying immediately under the layer of rods and cones, some light is reflected back into the viscus of the eye. It is this reflected light that makes retinoscopy, etc., possible. The reflected light, however, is in great part absorbed by the pigment of the iris and by the insensitive anterior portion of the retina. On the other hand, light entering the eye at a great angle would first strike the anterior retina and be reflected to the posterior sensitive part.

The possibility of the entry of oblique rays is reduced by sheltering the eye by the barriers of eyebrows, eyelids, eyelashes, cheeks and nose, and by the smallness of the pupil when light is intense. Under ordinary conditions one is not much troubled by scattered light in the eye.

Defects of the normal eye as an optical instrument.

(i.) The eye is not perfectly centred, *i.e.* the axis of the lens does not coincide with the axis of the cornea.

(ii.) The optical axis passing through the centre of the cornea and the centre of the lens does not coincide exactly with the visual axis passing through the centre of the cornea and the fovea centralis.

Neither of these defects interferes appreciably with the accuracy of vision

(iii.) Slight degrees of astigmatism are almost always present.

This is a "defect" of the cornea and is due to the fact that the surface is not uniformly part of a sphere. For example, the *top* of an egg is spherical, but a portion of the shell removed from the *side* would correspond in greater or less degree to the normal astigmatic cornea. Astigmatism is termed *regular* when the different meridians which cut the anterior surface present a gradual change in passing from one meridian to the other, the curvatures that are most different being at right angles to one another. The eye has, therefore, two anterior focal points, the one corresponding to the meridian of greater curvature nearer the eye than the one corresponding to the lesser curvature. The emmetropic eye has a certain amount of radial astigmatism, which is neutralised to a considerable extent by a *positive* axial astigmatism, *i.e.* a circular object appears as if compounded of two almost circular ovals in the same plane with the same "centre," but at right angles to one another. Such an astigmatism does not require correction by lenses.

Defects of the abnormal eye as an optical instrument.

In the normal or emmetropic eye, parallel rays, which in practice are those coming from a distance greater than 6 metres, are focussed in the resting state. Within 6 metres accommodation begins, and it increases as the object approaches, reaching its maximum at about 10 or 15 cm. This is called the *near point* of vision, and the point at which accommodation begins is called the *far point.*

The eyes of a considerable proportion of persons do not produce sharply focussed pictures within normal limits. The defect consists most commonly in the screen being (i) too far from the lens, or (ii.) too near the lens. In the first case the picture in the resting eye falls in front of the retina. This is the condition of *Myopia* or short sight. Divergent rays from near objects are focussed with little or no accommodation, and images are sharply defined when objects are well within the normal near point. But parallel rays cannot be focussed unless they are artificially rendered divergent before entering the eye. This is effected by the use of concave lenses. In the second case, parallel rays are focussed behind the retina in the resting eye. This is *Hypermetropia* or long sight. Such rays can be converged and brought to a focus by using accommodation, but the near point is reached while the object is still comparatively distant. To enable the eye to focus objects near at hand we strengthen its converging powers by interposing a convex lens.

Presbyopia is the result of a gradual diminution of the power

of accommodation through loss of elasticity of the lens as age advances, the retina retaining its normal position relative to the lens.

The Retina. (1) **The Mechanism.**

This, as we have seen, is the end-organ for vision. Our aim is now to endeavour to find out how light falling on this surface becomes an effective stimulus for the occipital cortex so that very slight differences in wave-length can be appreciated. There can be no doubt that the rods and cones are the actual irritable organs. Once they are stimulated, the disturbance is passed through their cell bodies to their processes in the outer molecular layer where the impulse passes across a synapse to bipolar neurones. These hand on the disturbance to the large oval ganglion cells whose axones form the stratum opticum. The axones are given a myelin sheath as they pass out of the eyeball and form the optic nerve.

Rods and Cones. As both rods and cones make connection in this way with the optic nerve, we have to consider these two types of receiving units. They differ in structure, in distribution, in their associations in the outer molecular layer and in function.

Structure. Briefly the rods, as their name implies, are columnar structures lying in contact with and normal to the pigmented layer of cells. Their inner portion is attached by a process to a small bipolar cyton whose other process gives off dendrites in the fifth layer (p. 277), several rods forming synaptic connection with one bipolar neurone of the inner nuclear layer.

Distribution. Rods are not found at the fovea and are associated with the visual purple.

Function. As more than one rod forms nervous connection with a single bipolar cell, their function cannot be that of exact minute definition. Further, as rods are not found at the fovea, the area of direct vision, they are not necessary for clear vision. Experiment shows that they are sensitive to light of low intensity, *i.e.* they are effective in twilight vision. When lighting is poor we readily detect movements. The rods, therefore, are easily stimulated by light of short duration provided it is not too bright. In the twilight everything appears dressed in shades of grey. Some greys are more easily seen than others. Examination of these greys in a good light shows that they are actually yellows and yellow-greens. From this we infer that the rods are colour blind, but have a lower threshold for light belonging about the middle of the visible spectrum.

Cones are shorter elements and are directly incorporated with their cyton, *i.e.* they are part of the first order neurones. They

form synaptic connection exclusively with one bipolar neurone each, *i.e.* the stimulation of one cone is not associated with the stimulation of any other cone till the disturbance has reached the cortex. They are thus fitted for point vision and are capable of transmitting fine detail if the light is not too intense. The fovea contains cones closely packed together; the surrounding area, cones and some rods. Then comes an area peripheral to this, with rods and some cones, and, finally, at the very edge of the effective sensitive surface very few cones are found. They are, therefore, the elements responsible for direct vision in good light, are capable of transmitting detail and are sensitive to all parts of the visible spectrum. They are not fitted for twilight vision and are not associated with the visual purple.

Fovea Centralis.. As this tiny depression in the retina is the screen on which inverted images of external objects are clearly focused under ordinary conditions, we must devote a little more attention to it. In this area the rays of light transmitted through cornea, aqueous humour, lens and vitreous body and "stopped down" by the iris, come otherwise undistorted into direct contact with the bases of the cones, which here are larger, more highly developed, and more rod-like than elsewhere. In order to get this freedom from the dissemination of light by the colloidal structures interposed in other parts of the retina, the nervous connections of the cones are led away from the fovea into the surrounding macula lutea. There are no blood vessels in the fovea, and this, also, contributes to exactness of vision. The cones, too, are so closely packed together that, in section, they become hexagonal, each flat side being in contact with the flat side of a neighbour. That is, in the fovea, there are essentially only two sets of elements, viz. cones and the cells of the stratum pigmenti.

Pigments. Several coloured substances have been described as being present in the retina. (1) In the outer layer, *i.e.* the layer lying between the layer of rods and cones and the chorioid coat, there is a pigment fucsin, which acts as a "damper" for the ethereal waves, *i.e.* the needles, prisms or plates of this pigment found in the processes of the cells of the stratum pigmenti prevent light from spreading by reflection from the outer limbs of rods and cones. (2) Visual purple, or rhodopsin, is found associated with the rods. In fact some investigators, like Edridge-Green, are inclined to consider that the main if not the sole function of the rods is to elaborate and secrete this pigment. Rhodopsin has been extracted from retinae and its photo-sensitiveness tested. Strong light bleaches it very rapidly, forming probably two substances, a principal one and an accessory one. These products of photolysis

are, in the eye, resynthesised into rhodopsin. Removed from the retina, the pigment bleaches at a rate which varies in velocity with the intensity and wave-length of the incident light and remains in its bleached state. That is, the rods or their adnexa are essential for the restoration process. (3) Other pigments, such as visual yellow and pigmented oil drops (amphibians, reptiles and birds) have been described, but their nature, distribution and function are not yet clear. It has been suggested that these red, yellow and green globules act as colour filters in much the same way as the coloured starch grains do in the Lumière process for colour photography.

The Retina. (2) **Effect of Light on it.**

When light falls on the sensitive surface of the retina physical and chemical changes occur.

(1) *Physical.*

(*a*) Pigment moves inwards (Boll and Kuhne, 1877).
(*b*) Cones retract (Angelucci, 1882).
(*c*) Outer parts of rods swell.
(*d*) Ganglionic chromatin is decreased.
(*e*) Electrical changes occur.

(2) *Chemical.*

(*f*) Retina develops an increase in H^+ concentration.
(*g*) Rhodopsin and fucsin, two pigments, are bleached,

and a physiological change is produced, whereby we see. Of these changes, (*a*) and (*b*), and probably (*c*) and (*d*) as well, are secondary products. They only occur if the nervous mechanism is intact, and may be produced by stimulation of either optic nerve by any means, *i.e.* they are caused by retinomotor impulses *from* the brain. That leaves three factors to be reckoned with in any explanation of the mechanism of the retina, viz. the electromotive force developed, the alteration in hydrogen ion concentration, and the bleaching of the pigments.

(1) *Electromotive Force.* Du Bois Reymond in 1849 noticed that alteration in the nature and intensity of illumination altered the potential of the retina as a whole. His work has been confirmed and amplified by Holmgren (1880) and later workers. In general, illumination causes a negative variation in the electromotive force of any point of the retina, *i.e.* a current would flow *in the retina* from an illuminated point to the rest of the structure. In other words, the illuminated part becomes zincative just like the injured part of any cell, the contracting part of a muscle or the momentary site of the impulse in a nerve. This is not strange when

we remember that the retina is primarily part of the nervous system, and that the electrical variations found are actually those accompanying the nervous impulse, and do not differ materially from those found by Adrian in the optic nerve itself. The change in E.M.F. does not occur exactly at the moment when the stimulating light falls on the retina. There is a definite latent period during which, it is presumed, some photochemical change takes place. In the case of peripheral vision, where rods are the main elements present, this latent period is occupied by the bleaching of the visual purple and the liberation of whatever substance is responsible for stimulating the rods, but in the fovea there is no visual purple to bleach. We can only assume at present that some substance is formed or altered by the incidence of light on the fovea, which in turn stimulates the cones. Edridge-Green has brought forward evidence that visual purple may diffuse from the surrounding rods into the fovea, be bleached by light there, and so stimulate the cones. Hecht and others have studied the relationship between the intensity and colour of the incident light on the one hand and the photochemico-electrical response on the other. With monochromatic light a geometric rise in the intensity of the light causes an arithmetic increase in the E.M.F. produced, *i.e.* if we increase the illumination from 60 candles to 3,600 candles we would double the potential developed. With coloured lights of apparently equal intensities, the yellows are more effective as stimulants when the general intensity is fairly high, and the greens when the intensity is low.

The following table from Fröhlich (1913) shows that, in order to produce the same E.M.F., it is necessary to have a greater intensity of blue light than of white light, and a much greater intensity of light when it is red than when it is blue.

TABLE XI

E.M.F. Millivolts.	Relative Intensity of Light.		
	White.	Blue.	Red.
Minimal	—	1	20
0·2	—	5	1,020
0·4	1·25	11·2	12,500
0·6	5	80	—
0·8	80	12,500	—

(2) *Chemical Changes.* We have seen that rhodopsin is bleached by incident light, with a velocity related to the intensity of the

light and to its colour. Proof has been given that Grotthus' law (*q.v.*) of photochemical action applies to this photolysis, and that the amount of light absorbed when light of various frequencies falls on the pigment is related quantitatively to the amount of chemical action produced. That is, if we constructed two curves, one showing the relative bleaching powers of light of various wave-lengths and the other showing the amount of light absorbed by the visual purple with light of the same wave-lengths, these curves would run a similar course. Further, a third curve produced by plotting wave-lengths against the intensity necessary to produce a minimal peripheral sensation of grey-blue would be of the same form as the other two. From this we infer that the amount of visual purple bleached to produce similar sensations is the same for all wave-lengths.

Peripheral Vision. When light strikes the peripheral parts of the retina it bleaches a certain amount of visual purple, producing two substances, either or both of which stimulate the irritable element. The general view is that the irritable elements concerned are the rods. Some people, however, are of opinion with Edridge-Green, that the rods merely produce the pigment and are necessary for its restoration, but have nothing to do with actual perception. That function they attribute exclusively to the cones which are present in decreasing concentration as the distance from the fovea increases. While admitting the validity of the evidence on which this theory is founded, one may draw other conclusions from it. It is difficult to shut one's eyes to the histological nature of the nervous connections of the rods (*q.v.*). It has been suggested that while the *cones* undoubtedly are stimulated by the bleaching of the rhodopsin, the *rods* might be stimulated by the decrease of the concentration of the pigment in their substances.

Acidity. Dittler (1907) showed that the illuminated retina became acid. This acidity decreased rapidly to the normal value when the eye became dark-adapted, *i.e.* remained in the dark.

Adaptation of Retina. The retinal mechanism has a rate of adaptation to a constant stimulus that is similar to that of the receptors for pressure, *i.e.* more rapid than that of the proprioceptors in general, *e.g.* muscle-spindle, and a good deal less rapid than that of touch, *e.g.* hair. A constant stimulus, therefore, ought to cease to be effective in a few seconds. That is, after a latent period the retina will send a burst of impulses to the cortex which will decline rapidly at first and then gradually fade away. This appears to be contradictory to our daily experience. But is it so? If you fix your eye steadily on this page and prevent your head and your eyes from moving, you will find that the print becomes rapidly

blurred, then remains in this blurred state apparently without change for a few seconds before involuntary movements of the body as a whole (respiratory or cardiac) cause a slight improvement in vision *pro tem.* The blurriness is primarily due to points of the image on the fovea alternating between adjacent cones and so allowing one cone to have its irritability restored while its neighbour is being excited. This rhythmic alternation of ability to function is a universal characteristic of biological systems and permits of a function being effectively carried out almost continuously over a reasonable period of time by any collection of units, *e.g.* cones, capillaries to an area, touch receptors, etc.

Movements of the Eyeball.

The eyeball lies at the front of the bony orbit, a cone-shaped canal with its apex directed backwards and pierced by the optic

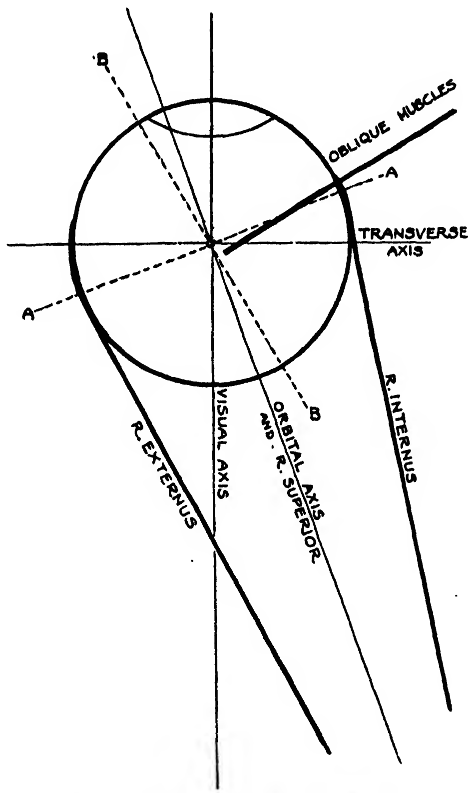


FIG. 74.—Diagram of extrinsic muscles of eye.

foramen. The antero-posterior or visual axis of the eye, *i.e.* the line passing through the centre of the cornea and the fovea makes an angle of about 20° with the long axis of the orbit. The move-

ments of the eyeball are carried out by the extrinsic muscles, which are six in number. Of these, four (called the recti) originate in a common tendon surrounding the optic foramen, and pass forward to be inserted a short distance in front of the equator of the eyeball. One is placed above (R. superior), one below (R. inferior), one on the outside (R. externus) and one on the inside (R. internus). The remaining two muscles are the superior and the inferior oblique. The former arises from the same tendon as the recti, and follows a course similar to but above the internal rectus. At the front of the orbit its tendon passes through a fibrous loop or pulley, and bends sharply back to be inserted in the eyeball about the equator. Its line of action makes an angle of about 60° with the visual axis. The inferior oblique arises from the inner anterior part of the orbital floor, and passes outwards

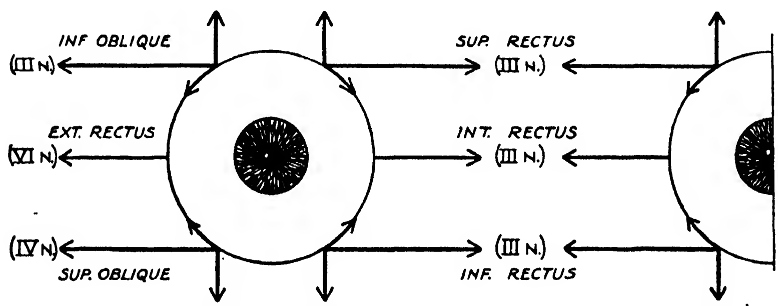


FIG. 75.—Diagram showing the directions in which the different external muscles of the eye rotate the eyeball.

and backwards to its insertion rather beyond the equator. It acts on the same line as the superior oblique.

All the movements of the eyeball are rotations round axes passing practically through the centre of the sphere, but it can be proved experimentally that rotation never occurs round the visual axis.

The internal and external recti rotate the eye round a vertical axis, and their action is unaffected by the relative obliquity of the visual and orbital axes.

The rectus superior acts along the line of the orbital axis, and its force can be resolved into two components, the one tending to rotation round a horizontal axis at right angles to the visual axis, the other tending to rotation round the visual axis itself in a counterclockwise direction (viewed from the front). In order to overcome the latter tendency, the inferior oblique acts simultaneously. Its force can likewise be resolved, one component tending to rotation round the horizontal axis at right angles to the visual axis, the second component tending to rotation round the visual axis, clockwise. The two rotations round the visual

axis counteract each other, the remaining two act as a couple and reinforce each other. The result of the combined action is to move the cornea vertically upwards. For movement upwards and inwards, for instance, the co-operation of a third muscle, the internal rectus, is required. The rectus inferior and the obliquus superior combine in an exactly similar manner to move the eyeball downwards (Fig. 75).

Binocular Vision.

1. **Focusing of objects within the far point.** We have seen that in order to focus near objects we employ accommodation. This is always accompanied by a movement of the eyeballs, causing the visual axes to converge from their parallel resting position towards the middle line. Such a movement is necessary in order that the images in both eyes may fall on the fovea centralis (Fig. 76). If this mechanism is defective, *strabismus* or squint results. The amount of convergence varies inversely as the distance of the object. The power of convergence is rather less than that of accommodation, for we can focus an object with one eye at a slightly shorter distance than when we use both eyes.

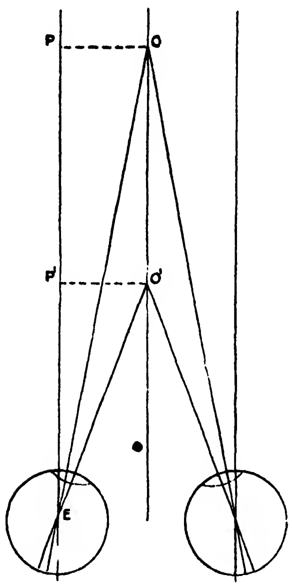


FIG. 76.—Convergence. O and O' are the far and near points respectively, and OEP and O'EP the angles of convergence.

It is only in the emmetropic eye that convergence and accommodation correspond exactly in amount. A hypermetropic person viewing a near object may require to use, say 4 dioptres of accommodation, whereas an emmetropic person uses only 2 dioptres, but both employ the same amount of convergence. Similarly an emmetrope may focus a near object, and if concave lenses are placed in front of his eyes he can continue to see the object plainly by increasing his accommodation sufficiently to neutralise their effect. The object still remains at the same distance, *i.e.* the convergence does not alter. Hence we see that accommodation and convergence are to some extent independent.

2. **Heterophoria.** In *strabismus* the deviation of the two visual axes is manifest, but the majority of people, with otherwise normal eyes, have a latent squint, only exhibited as an actual deviation when the eyes are dissociated by Maddox rods or other apparatus.

We have seen that the functions of accommodation and convergence are within limits co-ordinated, *i.e.* there is a movement inwards of *each* eye of 1 metre angle for each dioptre of accommodation. Now when the eyes are dissociated, and this may easily be done by holding a card sufficiently near one eye to prevent vision of the object by it and yet far enough away to permit of observation of that eye, and an object brought gradually from the far point (6 metres away) to the near point (7–17 cm.), accommodation and convergence do not keep pace with one another. The occluded eye will deviate about 3° or 4° outwards, *i.e.* at the near point all emmetropes are *exophoric.* As the object is taken further away from the eye the exophoric deviation of the dissociated eye tends to become less, and at a certain point, usually about 30–50 cm. away, no deviation is noticeable; that is, the extra-ocular muscles are perfectly balanced. The mechanism is, therefore, *orthophoric.* The latent deviation, or heterophoria, may be in a variety of directions, depending on which of the extra-ocular muscles are unbalanced. The directions may, however, for purposes of description, be resolved into four components pulling at right angles to the visual axis, *e.g.* upwards or downwards; inwards or outwards. The former two are deviations due to unbalanced action round a horizontal axis, and are termed *hyperphoria* or latent vertical deviations. Latent convergence is called *esophoria,* and latent divergence is *exophoria.* About three-quarters of the people with emmetropic eyes are esophoric, less than one-fifth are exophoric, and only about one in twenty is orthophoric. The value of the deviation is generally given as so many dioptres—the power of the lens required to correct it. The type of lens used depends on whether the object is to be "brought in" or "taken outwards." Consequently to correct heterophoria decentred lenses are used, convex lenses displaced against the deviation, and concave lenses with the deviation found by tests.

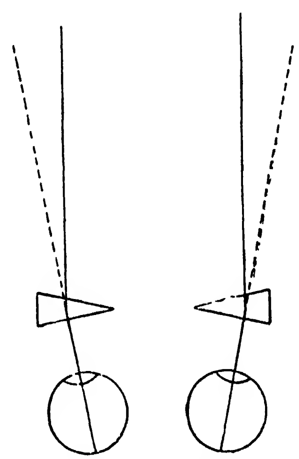


FIG. 77.—Artificial divergence of visual axes.

3. **Divergence.** In normal circumstances the visual axes never diverge, for they are in parallel adjustment for objects at infinity. Divergence can, however, be brought about artificially. Thus if we interpose prisms to render the rays divergent we can produce a corresponding divergence of the visual axes.

4. Visual Judgments. On account of the distance between the eyes, objects are viewed from a slightly different angle by each eye. This is readily demonstrated by looking at an object first with one eye and then with the other. It is particularly well brought out by two objects almost or completely in alignment, and the phenomenon persists at long distances. Each retina thus receives a slightly different picture, and we are furnished with a most important means of judging solidity and distance. Objects, seen with one eye only, have a very flat appearance. Shadows help materially in conveying impressions of solidity, and their significance may be illustrated in the interpretation of aeroplane photographs. In such photographs taken during the war there were numerous marks which might have either a positive or a negative solidity, *i.e.* they might have been either projections above the ground, such as machine-gun emplacements, or depressions, such as shell-holes. This could only be determined by the shadows cast, and the direction of the sun's rays was always marked on such photographs. Thus *A* is a projection and *B* a depression.

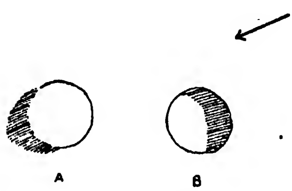


FIG. 78.—Shadows.

In judging very short distances, two eyes are an enormous advantage. Try to thread a needle with one eye closed. At rather longer distances this may be demonstrated by looking at a wall between which and the observer an object, such as the wire of an electric lamp, is suspended. Using one eye only and avoiding looking at the point of attachment to the ceiling, we will judge its distance from the wall very imperfectly, but with both eyes we can make an accurate estimation.

At long distances numerous external factors come into play. Perspective, light and shade and atmospheric conditions are of importance. Thus " visibility " may be good or bad, and will influence our judgments. At sea, where the surface is perfectly flat and the gradations of illumination change uniformly with distance, the untrained eye commits the grossest errors. The proximity of an object of known size frequently supplies a scale against which to measure the size and consequently the distance of unknown distant objects. The faculty of judging distances is poorly developed in the average man. A trained soldier or a big game shot can make incomparably more accurate estimations of distances for rifle fire than the novice. The same thing occurs with the expert golfer for certain distances.

5. The Stereoscope. The combination of two slightly dissimilar pictures to form an apparently solid object is illustrated by the

stereoscope. This instrument consists of two prisms or half lenses placed, with the thin edge inwards, about the same distance apart as the eyes. The dissimilar pictures P_1 and P_2 are fixed one in front of each lens, and a median screen cuts off each opposite picture. By means of this apparatus we obtain a single picture in the most pronounced relief, and situated apparently at P. (Fig. 79).

6. **The Visual Field.** When one eye is closed and the other fixed on a certain point the whole range of objects which can be seen without moving the eye or the head is called the *visual field.* The angle subtended by these objects is called the visual angle. With both eyes opened and fixed we command a greater range.

We may summarise the advantages of binocular vision as follows:

(i.) The visual field is increased.

(ii.) We receive impressions of solidity.

(iii.) We have a most important means of judging distances.

(iv.) The loss of one eye does not reduce us to blindness.

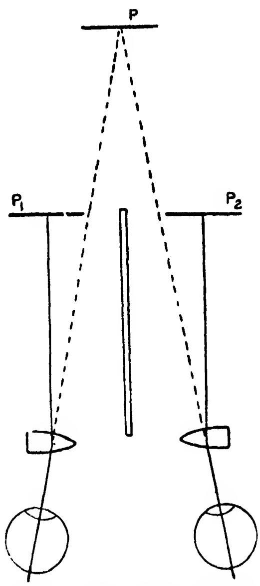


FIG. 79.—Diagram of stereoscope.

Analysis of Retinal Stimuli.

We have examined the eye as an optical instrument and discussed the mechanism whereby *an inverted image* of the external object is formed on the retina. We have also indicated that this image may be imprinted on the retina photochemically—a rapidly reversible reaction. It may puzzle the student as to why, the image being inverted on the retina, we see things right side up. Seeing is an acquired reaction, *i.e.* it implies learning. We do not *see* the image on the retina. What we do get is a series of nerve impulses coming to the cortex from one or more of our seven million cones. Just as with the other receptors, primary analysis takes place in the retina—number and position of cones stimulated, rate of bleaching of visual purple, etc.—and these changes, in the light of previous experiences, are analysed and interpreted in the cortex.

Purkinje Figures. Certain parts of our retina may be seen by ourselves, as shadows it is true, but nevertheless objectively. These are the blood-vessels and certain bundles of nerve-fibres. If, in a dark room, the eye is kept motionless, and light from a

candle, held near the temple, allowed to fall obliquely on the sclera, the shadows of the vessels are projected on to the retina. The eyes should have their accommodation fully relaxed and a suitable dark screen be placed in a plane parallel to the antero-posterior plane of the head. Under these conditions the vascular network of the retina appears, in a highly magnified form, as if projected on the screen. If the light is focused on the sclera by means of a biconvex lens, or if a brighter source of light is used, flashes of light following definite paths are seen. These flashes are produced every time a transparent leucocyte passes across the path of light. The interposition of a dark blue-violet light filter will make this phenomenon clearer. The red cells of the blood absorb the blue rays and appear as an almost continuous shadowy stream broken here and there by a leucocytic flash. By holding the breath one alters the rate of blood-flow, and so produces a definite alteration in the rate at which these flashes appear (Fig. 80).

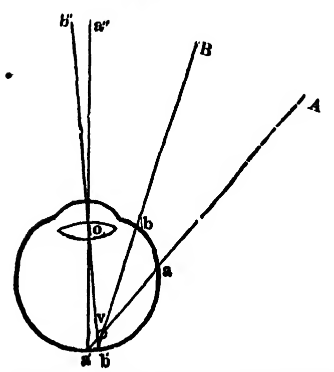


FIG. 80.—Diagram of the path of the rays of light in the formation of Purkinje's figures.

V represents a retinal vessel. When this is illuminated from *A*, a shadow is formed on the sensitive layer of the retina at *a'*. This is projected along a line passing through the optic axis and appears to come from a joint (*a''*) on the screen. On moving the light from *A* to *B*, the image of the vessel on the screen appears to move from *a''* to *b''*.

Arc Phenomenon. Under suitable conditions nerve bundles lying on the upper and lower borders of the temporal half of the macula and extending to the optic disc may be seen (Ellis). If one is looking at a distant street lamp (a doctor's *red* lamp serves well) in the evening, when it is possible to use a wall in shadow as a dark screen for projection purposes, two arcs of a bluish colour are seen, set with their concavities facing. The oval space between the arcs is filled with a bluish haze. If the distant light is feeble or poor in the more refrangible red rays, the arcs may be difficult to see and only the haze be perceptible.

Muscae Volitantes. These motes, which move steadily downwards as the eye is directed upwards, are parts of a diffraction pattern produced by substances in the vitreous body.

Ophthalmoscopy.

The interior of another person's eye may be viewed quite readily by suitable methods.

When we look at a person's eye the pupil appears perfectly black. Nothing can be seen of the interior because it is feebly illuminated compared with the outside world. If we could light

up the interior it would become visible by reflected light, just as we can see into a lighted room at night if the window is not covered with a blind. When we try to illuminate the interior of the eye, we find that we must interpose our head between it and the source of light in our attempts to peer into it. This difficulty is overcome by reflecting light from a mirror provided with a small central aperture, through which the observer can look. This instrument is called an *ophthalmoscope.* It may be used in two ways :

A. In *direct ophthalmoscopy* the mirror and the observer's eye are brought close to the observed eye, and the refracting media

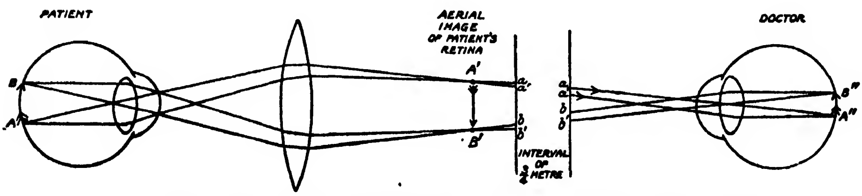


FIG. 81.—Diagram to show paths of rays from eye of patient (on left) to observer when the *indirect* method of ophthalmoscopy is in use (Hartridge).

of the latter produce a virtual image, erect and magnified, of the retina. The lens, etc., of the observed eye act in exactly the same way as a magnifying glass, the object being just inside the focus.

B. In *indirect ophthalmoscopy* the observer holds a convex lens in front of the observed eye, and places himself farther away. The interposed lens brings the rays leaving the observed eye to a focus between itself and the observer, who consequently sees an inverted image of the retina. This is real and magnified, the magnification depending on the lens used (Fig. 81).

FURTHER READING

EDRIDGE-GREEN. "The Physiology of Vision." Bell.
GOULDEN. "Refraction of the Eye." J. & A. Churchill.

SECTION IV: TRANSPORT

CHAPTER XXII

THE BLOOD

INLAND TRANSPORT SERVICE

"If they flourish not, a kingdom may have good limmes, but will have empty veines and nourish little." BACON.

We have seen reason to consider the animal body as a country containing numerous cell-communities, each busily engaged on its specific staple industry and connected with one another and with the seat of government by an extremely efficient means of communication—the nervous system. Such a country, on account of its complex nature, must have a system of transport. Raw materials from outside must be brought in, and some means must exist for sorting out the imports and forwarding the suitable ones to the appropriate cell-communities, etc. It is convenient to carry still further this simile of a country.

It is obvious that some imports may arrive from overseas ready for use and have only to be handed to the distributors for repacking and transmission to the consumer. Others have to undergo some change before they can be transported inland. That is, *there are two classes of raw material* arriving at the same port, viz.: gas and liquid-solid food. By a mechanism which will be considered in a subsequent chapter, the gas is diverted to one basin of the harbour, while the food material is passed to a canal—the alimentary canal. The gas is sent directly to the inland transport service, while the food material is sent to a series of factories where it undergoes partial manufacture and is repacked in smaller containers, before being handed to the same inland transport service. Just so, iron ore may be shipped to the Clyde, from which it passes through a series of factories, in which it is partially purified, smelted, etc., and then sent as pig-iron, say to Sheffield, for final treatment, before being distributed in a useful form over the country. There are, therefore, two forms of transport, which we may term external and internal. As all material has finally to be carried by the inland transport service and as the

amount of traffic on this system to some extent controls the rate of importation, it will be convenient to direct attention to it in the first place.

Inland Transport—The Blood

The blood has been called the liquid tissue of the body. On two counts this is a misnomer. Firstly, it tends to detract from the liquidity of the tissues in general. Further, blood cannot rightly be considered a tissue at all. No doubt a very pretty picture could be drawn of blood and its containing membranes as a tissue, clotting, as other tissues clot, on death, but when the facts are examined they do not bear out such an idea. The evidence too, culled from comparative studies of the development of a circulatory system, is all at variance with the liquid tissue theory.

1. Development.

Much may be learned from a study of the evolution of any system. Material exists for such a study in Comparative Physiology.

(*a*) Unicellular organisms require no circulatory system. Their imports go direct to the sole factory of the place. They may be landed at any part of the coast and are at once acted on. What is suitable is accepted, the residue is rejected or left untouched. Examination of a unicellular animal leads to the conclusion that the cell contents are in a state of constant motion. Water, every now and then, is engulfed, passes more or less directly through the organism, and is excreted, carrying with it the by-products of cell activity.

(*b*) Some invertebrates have an open cœlomic system. Their more complex structure necessitates the production of a current of fluid so that material may reach the inner cells. That is, some of the water in which the animal lives is passed by means of canals to the different parts of the body. The fluid is kept in circulation by the rhythmic contractions of whip-like processes called cilia (*q.v.*) The ciliary waves force the water through the tubes into the lacunæ of the tissues. Such a system is difficult to control. It is dependent on the nature of the bathing medium. It carries the possibility of constant changes in the salt content of the cells of the animal. Any change in the environment will be passed on to the cœlomic fluid and at once reflected in the cell. It demands constant adaptation on the part of the organism, and thus it is not economical (cf. our system of canals).

(*c*) Higher invertebrates and the vertebrate *amphioxus* have

a closed system in which the fluid passes through tubes capable of rhythmic contractions.

(*d*) The vertebrates have a closed vascular system, with the advantages of ease of control and freedom from constant adaptation. It is the most economic system of transport known.

When the sea animals crawled on to land and became breathers of air, they included a certain proportion of the sea-water in their vessels. By the alteration in surface tension caused by the exchange of a protoplasm-water interface for a protoplasm-air interface their open cœlomic system automatically closed (cf. camel's hair brush experiment, Part II.). The vertebrate has, therefore, a fluid in its vessels having a composition similar to that of the sea from which originally it came (see salts of plasma, p. 310). This is a very pretty theory. It cannot be considered as proved any more than the hypothesis of evolution, but in the same sense both fit in with certain facts.

2. Function.

(*a*) The blood-stream conveys materials for building, repair and renewal of tissues, as well as oxygen, water and potential energy to all parts of the organism.

(*b*) It removes the waste products of activity including carbon-dioxide, which would paralyse function if allowed to accumulate.

(*c*) The carriage of chemical substances (hormones) from the organs in which they are produced in order to influence the activity of other organs may be considered as the co-ordinative action of the circulation.

(*d*) The movement of blood aids in the regulation of the temperature of the body (Chap. XXXII.).

(*e*) It plays a very important part in the defence of the organism against parasites, etc.

(*f*) The preservation of the H-ion concentration of the body is principally a function of the circulating fluid (Chap. XXXI.).

(*g*) It maintains the water and salt content of the body at a certain level.

3. Composition.

Since the function of the blood is to act as common carrier to all the parts of the body, it has to convey food material from the digestive organs and oxygen from the lungs to the tissues. From these it receives in exchange their waste products, viz. results of nitrogenous metabolism (urea, etc.), CO_2 and H_2O, and carries them away to the excretory organs, kidneys, lungs, skin, etc., by which they are eliminated. It is therefore evident that the

composition of the blood must vary from time to time and from place to place, according to the activity and the function of the organ which it is traversing. The cells of the body are adjusted to respond to very minute changes in the composition of the blood and, therefore, changes are kept within infinitesimal limits.

The term blood or whole blood is usually applied to the fluid content of the vascular system, plus the formed elements suspended in it.

I. Fluid or Plasma

(*a*) **Physical Characters.**

(i.) *Colour*, light straw.

(ii.) *Opacity*, practically transparent.

(iii.) *Specific Gravity*, about 1,030. The specific gravity is lowered after a meal because of the dilution of the plasma by ingested water. Conversely, exercise and profuse perspiration cause a slight increase in the specific gravity on account of the loss of water. Variation in activity will therefore produce a diurnal variation—a decrease during the day and an increase during rest at night. The night worker, of course, has this reversed. It varies greatly in individuals, so that a figure which is normal for one person may be pathological for another.

(iv.) *Viscosity.* At body temperature (37° C.) plasma has a viscosity about twice that of distilled water, *i.e.* 1·7–2·09. Salt solutions have almost the same viscosity as water. This factor is due to the emulsoid colloids present (*q.v.*), one of which by forming a gel under certain conditions may produce so great an increase in viscosity that the flow of plasma may be entirely stopped. The plasma is then said to clot (see fibrinogen and also viscosity of blood).

(v.) *Reaction.* Plasma turns red litmus blue and therefore has an H-ion concentration under 10^{-7}. It is acid to phenolphthalein and therefore has an H-ion concentration greater than 10^{-8}. Exact determinations have shown that the *p*H of plasma is 7·4, *i.e.* just on the alkaline side of neutrality. This alkalinity is due to the presence of sodium bicarbonate (see below).

(vi.) *Colligative Properties.* It is of academic interest to ascertain the values of the vapour pressure, osmotic pressure, and depression of the freezing-point of plasma, and many attempts have been made to correlate changes in these values with the symptoms of disease. As we have seen in studying the colligative properties of dilute solutions (Chaps. V. and VI.), the temperature at which the determinations are made is of great importance. Grollman has determined the values for "separated" plasma

saturated with CO_2 at its tension in alveolar air and at body temperature (37·5° C.). For dog's plasma he finds the depression of the freezing-point = 0·61° C., for vapour pressure 48·1 mm. Hg, and *calculates* from these an osmotic pressure of 8·2 atmospheres. This value for the osmotic pressure is due very largely to the crystalloids present, as shown by separating colloids from crystalloids by the process of ultrafiltration (*q.v.*). It is then found that over 8 atmospheres pressure is given by the diffusible salts, leaving only about 0·06 of an atmosphere, *i.e.* 46 mm. Hg., due to the colloids.

That is, the osmotic pressure of separated plasma as taken by an ordinary osmometer with a semi-permeable membrane or by the depression of the freezing point is almost the same as that exhibited by a 0·9 per cent. solution of sodium chloride. It varies with the diet and with the amount of fluid ingested. If the kidneys are not functioning properly, so that the products of metabolism are not eliminated with sufficient rapidity, the osmotic pressure will rise.

The student cannot guard too carefully against the errors of considering that (*a*) the osmotic pressure of plasma is due to the presence of 0·9 per cent. NaCl in it, and (*b*) that the figure given even approximates to the proper value of the osmotic pressure *in the blood vessels.* These vessels are permeable to salts in solution, and, therefore, the *true* osmotic pressure of plasma must be due not to crystalloids, but to colloids. Further, plasma divorced from its formed elements, especially the red corpuscles, is very different from "true plasma," which is plasma removed with such precautions that for any given tension of CO_2 it is in equilibrium with the cells of the blood.

(vii.) *Refractive Index* (see p. 273). The refractive index of plasma depends primarily on the amount and nature of the proteins present. Its variations are governed by practically the same factors as are responsible for the variations in specific gravity.

(*b*) **Components.** (i.) **Colloids.**

The major colloidal constituents of plasma are protein in chemical nature. These proteins are :

(α)	Albumin . .	2·5 per cent. circa.
(β)	Globulin . .	3·8 per cent. circa.
(γ)	Fibrinogen . .	0·15–0·6 per cent.

(α) Albumin, probably a mixture of three albumins. At least it is possible by careful heating to discover three separate coagulation temperatures.

(β) Globulin is similarly a mixture of two or more globulins. Globulins are insoluble in distilled water, but soluble in dilute salt

solutions. They therefore require to have a certain concentration of electrolytes present if they are to remain in solution. They may be partially separated from albumin by dialysis. When the salt content of plasma is forced below a concentration of about 0·2 per cent., the globulins are almost completely precipitated. In the blood-stream they function to a great extent as regulators of the amount of NaCl present. It is of importance that this fact be thoroughly grasped. Where the amount of globulin in the blood is increased, the chloride content increases, *e.g.* in pneumonia. In patients with this infection, as well as in cases of chronic nephritis and syphilis, the total protein content of plasma is decreased and the globulin content increased both absolutely and relatively. In mild infections and in chronic septic conditions the total amount of protein present remains normal, while the amount of globulin and of chlorides shows a marked increase. NaCl held by globulin acts as if adsorbed, *i.e.* exerts no osmotic pressure. Globulin is precipitated by an increase in hydrogen ions. It is specially sensitive to CO_2.

(γ) Fibrinogen, a globulin formed in the reticulo-endothelial cells of the liver (Faludi).

The osmotic pressure in the vessels is due to proteins. They are also responsible for the viscosity of blood. The removal of the proteins from blood lowers its viscosity to very little greater than that of water. After extensive bleeding water pours into the vessels from the tissues through the lymph, and the specific gravity, viscosity, etc., drop. The blood ceases to be an efficient carrier. Bayliss found that the injection of a non-toxic emulsoid colloid would restore normal conditions for sufficiently long a time as would enable the cell-factories, especially the liver, to manufacture new blood proteins from the amino acids in the blood. The most efficient sol, he found, was a solution of gum arabic in Ringer's solution. The story of this discovery as told in his monograph is one of the most interesting side-lights on the medico-scientific work of the war.

Clotting

The main use of fibrinogen lies, not in its viscosity or in its osmotic pressure, but in its property of changing from a sol into a gel. The clotting of plasma prevents the loss of blood and keeps the blood channel free from any angularities. The evolution of the knowledge of the process of clot formation has been very slow, and even now the physico-chemical reactions involved are anything but well understood.

Mammalian plasma, if left standing in a tube exposed to the air, clots to a jelly in 2 to 10 minutes. The gel has the same volume as the sol, and no heat is evolved in the process. This is a process common to most emulsoid colloids. In about half an hour the clot contracts and expresses a clear straw-yellow liquid, the *serum.*

i.e. Plasma = Clot + Serum.

This process whereby the fluid content of the gel is decreased is common to gels and is called *syneresis.*

Historical.

Observers at first thought that living tissue had a restraining influence. They noticed that in the living tissue the blood did not clot. Lister formed a living test-tube by ligature of the aorta and showed that the blood did not clot until a foreign body such as glass was introduced. This demonstrated that clotting was not due to (1) removal from living vessels, (2) stoppage of the circulation, (3) cooling or (4) to contact with clean air. This latter fact has been confirmed by the observation that blood clots as readily in a vacuum as in air. Nor is clot formation due to cooling. As a matter of fact reduction of temperature lengthens the time taken to produce a clot and, if the plasma is cooled sufficiently, may prevent it altogether.

The classical work on blood clotting was performed by Andrew Buchanan, Professor of Physiology at Glasgow, who gave explanations of the process to the Glasgow Philosophical Society, in March, 1844, and February, 1845. Using hydrocele fluid from the *tunica vaginalis* of a horse, he showed that it would clot if to it were added a drop or two of blood, of clot washings, of serum or of tissue juice. He compared the process to the curdling of milk by rennin and considered that the white corpuscles or leucocytes were the active agent.

In 1861 Schmidt, who had devoted some thirty years to the work, proved that:

(i.) Fibrinogen, the precursor of the clot, was a globulin in the plasma.

(ii.) There was a fibrin-former in plasma, in serum and in clot washings.

(iii.) He later showed that this fibrin-former (now called thrombin) did not exist as such in the blood, but only appeared after treatment with a fibrin-ferment or -kinase. That is, the clotting scheme as he left it appears as follows (modern names):

1. Thrombokinase + Prothrombin → Thrombin.
2. Thrombin + Fibrinogen → Fibrin (Clot).

Arthus and Pagès in 1890 showed the need of calcium in the clotting process.

Hammersten confirmed this, and found that the calcium acts on the prothrombin producing active thrombin.

This still does not explain why blood does not clot in the vessels, so physiologists had to postulate the presence of some substance in the blood which would prevent the calcium from activating the prothrombin. This hypothetical substance they called anti-prothrombin. When a vessel is ruptured and blood comes into contact with the tissues or into contact with disintegrating blood-cells, it takes from them a substance which neutralises the inhibitory action of the anti-prothrombin and so allows the calcium to act. This substance was, at that time, believed to be a ferment, or kinase, and was, therefore, named "*thrombokinase*." That is, in all cells there is a large or small quantity of thrombokinase, which is the trigger setting off the whole clotting process. It is now known that this substance is not a ferment or kinase. Some people, therefore, prefer to call it *thromboplastin*.

That is, we have in the blood-stream *prothrombin*, *anti-prothrombin*, *fibrinogen* and *calcium salts*. In order to start the process of clotting, *thrombin* must be liberated from its precursor. This duty falls on *thromboplastin*, a substance present in all tissues and, in vertebrates, also in the white corpuscles, and perhaps in the platelets (*q.v.*). Can we form a picture of the process? Examination of plasma during the process of clotting on a microscope slide with dark-ground illumination shows how, on the introduction of a drop of serum, tissue juice, etc., fine needles of fibrin seem to radiate *from the added fluid*. These liquid crystals pack together to form a felt-work of fibrils of fibrin—a very similar process to that studied in an earlier chapter, viz. myelin forms of lecithin (p. 109).

Pickering and Hewitt have produced evidence to show the lines on which the clotting process runs. Their scheme may have to be modified in detail as our knowledge of the physical chemistry of colloidal systems like blood increases, but it gives a plausible explanation of all the known facts. According to their theory, blood contains *all* the elements necessary to form a clot, but it maintains its fluidity because of the presence of a protective substance. That is, we have two substances—a sol and a solution, fibrinogen and thrombin respectively, which would interact to form a liquid-crystal complex—a gel, but for the presence of an inhibiting body. This inhibiting body may be a substance acting in a manner similar to the protective emulsoids (Chap. VIII.), or it may interfere with the process of gelation like certain solutes

(Chap. VIII. and Part II.), or, according to Tait, it may be the membrane surrounding one type of the cells in the blood (thigmocytes or platelets). The addition of a phospholipin, thromboplastin, with the aid of the calcium of the blood, overcomes the protective power of the anti-prothrombin, neutralises the inhibitory solute, or causes the membrane of the thigmocyte to be ruptured, depending on which view one adopts.

(1) **Surface.** Howell presents experimental proof that the thromboplastic substance is either *kephalin* or some other phospholipin of similar composition and structure. Pure kephalin has not been prepared in sufficient quantity to allow of complete analysis, but its molecule probably consists of :

Two fatty acids $\begin{cases} \text{Stearic (saturated)} \\ \text{Linolic (unsaturated),} \end{cases}$

+

an amino alcohol, $NH_2\text{-}CH_2\text{-}CH_2\text{-}OH$
(amino ethyl alcohol),

+

a glycero-phosphoric acid.

It is optically active, rotating the plane of polarised light to the right (p. 126).

The main point of interest for us apart from its presence in all tissues is its colloidal and fatty character. It is an extremely hydrophilic emulsoid with a strong negative charge. On exposure to air it readily absorbs oxygen (a property dependent on its unsaturated fatty acid) and undergoes partial decomposition.

The first step in the formation of the clot is the liberation of kephalin or some such thromboplastic substance which, by interaction with calcium soaps or other colloidal calcium complex, offers water-wettable surfaces to which the thigmocytes (Tait) or other blood cells may adhere and over which they will spread. The spreading process causes mechanical rupture of the cells, with the liberation of thrombin (a proteose) from their contents. Then, as we have seen, thrombin acts on fibrinogen and a clot of fibrin is produced.

That the introduction of a hydrophilic surface can initiate the process of clot formation can easily be demonstrated. Almost any hydrophilic substance with an *effective surface* (p. 71) will answer as well as thromboplastin, *e.g.* powdered glass, quartz particles, bubbles of carbon-dioxide gas, etc. Emboli (bubbles of gas) are apt to cause clotting in the vessels where they stick, *i.e.* in the smaller vessels. Other foreign substances whose surfaces can be wet by plasma have the same effect.

It is worthy of note that kephalin particles of a size just visible

under an oil-immersion microscope are capable of producing intravascular clotting when injected into the blood-stream, while powdered glass or quartz particles of apparently the same size are without effect. Coarser glass suspensions are necessary to produce the effect. Some other factor in addition to surface must come into play or kephalin particles must have a wrinkled surface.

(2) **H-ion concentration.** This view of the process of blood-clotting has been challenged by Pickering and Hewitt and by others as an incomplete picture. It does not account for the fact that, if due precautions are taken, birds' blood, which contains *all* the constituents necessary for the formation of a clot, remains liquid for a considerable time after removal from the vessels. The addition of a mere trace of acid causes immediate and extensive clotting. From this one may infer that in avian blood the formation of a clot depends primarily on the attainment of a definite hydrogen ion concentration. That this inference is justifiable, not only for birds' blood, but also for mammalian blood, is indicated by experiments where blood was prevented from coagulating by the liberation of alkali in it. Tri-calcium phosphate, the salt of a weak acid, H_3PO_4, and of a strong base, $Ca(OH)_2$, dissociates in aqueous solution as shown on p. 68, liberating -OH ions, and so tending to reduce the hydrogen ion concentration of the solution. Thus :

$$Ca_3(PO_4)_2 \rightarrow 3Ca^{++} + 2PO_4^{---}$$

$$6H_2O \rightarrow 6H^{+} + 6OH^{-}$$

$$\rightarrow 3Ca(OH)_2 + 2H_3PO_4$$

(strong base) (weak acid)

Now, when tri-calcium phosphate is added to mammalian blood, some of the salt dissociates, liberating alkali, and so preventing clotting. Further, it has been found that fibrinogen in the presence of the serum proteins, with their non-diffusible calcium, readily forms an insoluble complex gel with a phosphatide *in a slightly acid medium.* Confirmation of the part played in this process by the pH of the blood is deducible from Kugelmass' investigations on the change in pH of the blood during coagulation. Blood has a pH of 7·4, but the optimal pH for the process of clotting is 7. From such experiments, the conclusion may be drawn that alkali is the factor in the blood which prevents intra-vascular clotting. As we shall see later, blood has a considerable alkali reserve (buffering power), so that a tendency to reduce the hydrogen ion concentration is restricted within narrow limits.

(3) **Calcium.** During coagulation the electrical conductivity of

the blood decreases markedly, *i.e.* the concentration and mobility of ions has been reduced. From a study of the form of the curves produced by plotting the rate of diminution of electrical conductivity against time, and the rate of fibrin formation against time, etc., one arrives at two conclusions, viz. (*a*) that the process is auto-catalytic (*q.v.*) and is in two stages; and (*b*) that calcium is necessary only in one of these stages, *i.e.* in the liberation of thrombin from its precursor.

Stewart and Percival have carefully examined the part played by calcium in the coagulation process. This metal exists in the blood in three forms, viz. ionic, molecular and colloidal. The last is non-dialysible, while the two other forms readily pass through a dialysing membrane. According to these investigators, it is the non-diffusible calcium that is effective in the formation of a clot. Ionic calcium seems definitely to inhibit the process.

Certain substances by preventing the calcium from acting on prothrombin prevent clotting. One of these, called heparin by Howell, arises in the liver. Certain salts act on the calcium. For example, oxalates, fluorides, etc., form insoluble calcium salts, and when they are added in sufficient quantity they will cause the precipitation of the major portion of the total calcium of the blood. Citrates, on the other hand, reduce both the ionic and the non-diffusible calcium, and if enough citrate is added, all the calcium of the blood will be in the non-ionised but *diffusible* form. The addition of a soluble calcium salt to oxalated or citrated blood causes, in time, an increase in the non-diffusible calcium. When this latter reaches a certain concentration coagulation occurs.

(4) **Platelets.** The platelets (*q.v.*) have recently received renewed attention as clot-formers. Bedson and others have noticed that in whole blood, clotting seems to start at the platelets, *i.e.* they may initiate the process. Further, when the platelets are excluded, a clot may still be formed, but it does not undergo typical retraction and lacks firmness. If such a clot is plugging a wound it is very easily dislodged. A good clot normally formed in the presence of sufficient platelets would have a considerable tenacity, holding firmly to the edges of the wound and so knitting these edges together. Bedson attributes to the platelets the production of thrombokinase (cf. Tait, above).

Most of the work mentioned above has been done *in vitro;* that is, blood has been collected into a vessel, or plasma has been separated from the corpuscles by centrifuging, and allowed to clot under observation. Such experiments may lead to quite a mistaken idea of the process as it occurs *in vivo.* When a blood vessel is punctured and the blood allowed to flow to the surface

of the skin, one finds that the platelets gather at the edge of the wound, forming a gelatinous annular mass. As this mass grows in size a fine interlacing network of fibrin appears in it, with platelets at the nodes or crossings of the net. White cells are incorporated, and as the network becomes more felt-like, red cells are entangled and imprisoned. Sometimes the clot seems to be merely a mass of closely-packed platelets, leucocytes and erythrocytes (with very little fibrin) plugging the wound.

Anti-coagulants. The removal of any one of the participating substances from the sphere of activity will naturally prevent clotting. Let us take the process step by step.

(1) Liberation of thromboplastic substance is prevented by carefully drawing the blood through a paraffined tube into a vaselined or waxed vessel. If there is no contact with a water-wettable surface, there will be no clot. One of the old experimenters found that blood placed with due precautions on a *nasturtium* leaf would remain liquid for long. It is well known that water runs off the leaf of this plant.

(1*a*) Platelets may be reduced below their effective concentration by anti-platelet serum. In this way (and in purpura in man) clotting power may be poor, wounds hard to heal, and capillary bleeding dangerous.

(2) We have dealt with the inactivation of the calcium above.

(3) Thrombin may be rendered ineffective by the action of substances such as *hirudin*, a proteose prepared from the buccal glands of the medicinal leech. It is injected in blood-pressure experiments to prevent clots from forming in the arterial cannula.

(4) The fibrin may be removed as rapidly as it is formed by agitating the blood with glass beads or whipping it with twigs. The sticky fibrin adheres to the beads or twigs and may be removed. These methods and some others are summarised in Table XLI.

Differing from all the above anti-coagulants which act whether injected into the blood-stream or added to shed blood, are those which prevent clotting only when injected. Snake venom in amounts as small as 0·00001 gram per kg. suffices. Commercial peptone (mixture of proteoses and peptones) injected into the circulation in the proportion of 0·3 gram per kg. produces a non-coagulable blood for an hour or so. This class of coagulant seems to act by stimulating the liver to manufacture heparin. Peptonisation and the injection of venom may cause some alteration in the state of the thigmocytes. This has not been investigated.

Coagulants. Extracts of organs rich in kephalin, *e.g.* thymus, testes, lymph glands, produce intravascular clotting.

TABLE XLI

Non-clotting Plasma

Nature of Plasma.	Reason.	To cause Clotting.
Whipped (Defibrinated).	Fibrin removed as soon as formed by whipping with a bunch of twigs.	Add fibrinogen to whipped plasma (or blood).
Salted. $\frac{1}{2}$ sat. with $(NH)_2SO_4$.	Fibrinogen precipitated.	Dilute with water and so reduce concentration of electrolytes.
Cooled to 0° C.	Cooling slows the physico-chemical changes which are optimum at about 40° C.	Warm.
Oxalated. Addition of soluble oxalate.	Ppts. calcium of all forms.	Add soluble calcium salt.
Citrated.	Reduces concentration of non-diffusible Ca.	Add soluble calcium salt.
Addition of $Ca_3(PO_4)_2$.	Reduces H-ion concentration	Add acid.
Hirudinised.	Combines with thrombin.	Add thrombin.
Peptonised.	Stimulates production of anti-prothrombin.	Dilute to decrease concentration of anti-prothrombin, or add tissue extract or kephalin or pass in CO_2 gas.

Similarly, certain snake venoms which contain large quantities of thrombin cause clotting of the blood in the vessels and rapid death.

Components. (ii.) **Crystalloids.**

There is nothing more remarkable than the maintenance of a fairly constant concentration of crystalloids in plasma, under the most varied conditions. As we have seen, this is due in great part to the salt- and water-holding power of the colloidal constituents, especially of the globulins. Bunge, in his handbook of physiological and pathological chemistry (1889), suggested that as the notochord and branchial clefts were legacies from forebears who had lived in the sea, the high sodium chloride content of mammalian blood might also be an heirloom from marine ancestors. No doubt the circulation fluid of marine animals with an open cœlomic system is sea water. It is held by many observers, that when the ancestral form of vertebrates acquired a closed form of circulatory system, the fluid shut in was sea water. Analysis shows that while the concentrations of crystalloids in

plasma and in sea water are not similar, yet there is a remarkable resemblance in the proportions of the main salts present in both.

	Na	K	Ca	Mg
Serum (defibrinated plasma) .	100	6·69	2·58	0·8
Ocean	100	3·66	3·84	11·99

The similarity in proportion is not very striking because the figures given are from analysis of the ocean as it is to-day. What we should have is the analysis of the ocean in prevertebrate days.

Not only has the concentration of the salts of the sea undergone change, but alterations have taken place in the proportion of its constituents. Sodium and magnesium have increased in concentration and are still increasing. Material lixiviated from river beds, etc., is rich in those salts.

On the other hand, potassium and calcium have decreased. The formation of soil leads to the abstraction of potassium from the river water. Water evaporated from the ocean contains potassium in not inconsiderable amounts. The rain falling in the region of Caen is responsible for an annual increment to the land of 1·23 tons of potassium per square mile. Rivers discharge more calcium into the ocean than they do of sodium, magnesium or potassium, and yet the concentration of this element appears to be fairly steady. The cause for this lies in the formation of rock-beds of gypsum ($CaSO_4$) and limestone ($CaCO_3$) and in the formation of calcareous skeleta ($CaHPO_4$).

From the study of fossil seas and of lakes surrounded by pre-Cambrian formations as well as from other geological considerations it has been decided that the ocean of Cambrian days had a ratio of salts somewhat as follows.

	Na	K	Ca	Mg
Present day	100	3·6	3·9	11·9
Cambrian	100	6·0	3·0	2·0
Serum	100	6·6	2·5	0·8

The salt content of the plasma is regulated by the kidneys. It is interesting to note that while the blood is Cambrian the tissues are decidedly pre-Cambrian in their salt content, *e.g.* Muscle. Na = 100, K = 400, Ca = 9·3, Mg = 26·4. This may be in part accounted for by the adsorption of salts by the protoplasmic colloids. Of these salts, one of the most important is sodium bicarbonate on account of its power of neutralising acid. This has been termed its "**buffer**" value—a term which, although faulty, has crept into the writings of physiologists and clinicians and seems firmly ensconced there because it is handy. As Prof. Bayliss points out, a railway buffer absorbs shock but not the engine, while $NaHCO_3$ absorbs the acid.

The amount of $NaHCO_3$ present in plasma has been called the **alkali reserve of the body.** How does it act? The addition of acid to bicarbonate may be represented by the equation

$$NaHCO_3 + HA = NaA + H_2O + CO_2.$$

Until nearly all the bicarbonate has been acted on by the acid, no increase in acidity can be detected. This is a mechanism of great value to the organism. Acids are constantly being produced in the tissues, especially in muscle. Unless the organism had an alkali reserve, the concentration of hydrogen ions would so increase after muscular exercise, for instance, that a serious derangement of metabolism would ensue (see Preservation of Neutrality, Chap. XXXI.).

II. Formed Elements

The formed elements borne by the plasma have a volume about equal to that of the plasma and weigh about the same amount.

	Plasma.	Corpuscles.
Volume . .	52–48 per cent.	48–52 per cent.
Weight . .	3	2

This may be determined by the haematocrite (see Part II.) or by an ingenious method due to Stewart. He made use of the fact that the presence of corpuscles reduces the electrical conductivity of plasma in proportion to their number.

The Corpuscles

The blood corpuscles are of three kinds: (i.) coloured corpuscles or erythrocytes; (ii.) colourless corpuscles or leucocytes; and (iii.) blood platelets. The colourless corpuscles have been mentioned already in Chaps. XII. and XVI., and two types of them have been portrayed in Figs. 36 and 37.

The **blood-platelets** (*q.v.*) are oval, colourless, refractile discs, varying somewhat in size, but with an average measurement of 3μ in their longest diameter. There are from 200,000 to 300,000 of them in a cubic millimetre of blood. There is some doubt as to the title of the platelet to be considered a cell. It has no nucleus or chromatin material, and seems to consist of a homogeneous matrix in which highly refractile granules are embedded. These granules stain with *basic* stains (*q.v.*) like cresyl blue, and so are called *azurophil* granules. Many haematologists are of the opinion that the platelet is a complete cell budded off from the giant cells or megakaryocyte of the bone marrow. Others consider that they are fragments of cells or are the nuclei from young erythrocytes, or even are some of the protein matter of the plasma gelatinised. Whatever their origin, their function in the initiation of clotting and in the formation of a firm thrombus is unquestioned (*q.v.*).

The **erythrocytes** are the *carriers*, submerged barges into which are packed the oxygen for the tissues and some of the carbon dioxide from the tissues. They are born in the red bone marrow.

The young corpuscle is called an *erythroblast* and has a nucleus. It may live and die in its place of origin. In that case the valuable constituent of the pigment, the iron, is retained by the marrow and used in the construction of other cells. Most of the cells, however, do not remain in the bone marrow. Their nuclei undergo pyknosis and the enucleated corpuscles pass into the blood stream on their way to the liver and spleen, where they are alleged to be destroyed after 10 to 40 days. Those which die by the way are taken out of the circulation by the spleen. Economical Nature in this way makes use of the dying erythrocyte as a beast of burden.

In the next chapter we will consider the function of the red cells in the transport of the respiratory gases, and later will give consideration to the part they play in the preservation of a constant pH in the blood. There are one or two interesting physical problems in connection with the shape, structure and composition of the red cell which must first be considered. Why should the mammal (except *camelidae*) have circular biconcave discoids ?

Shape, Volume, etc., of Red Cells.

The 5,000,000 ($\pm$ 1,000,000) of these cells found on the average in every cubic millimetre of healthy human blood vary in diameter and in thickness with variations in the composition of the plasma in which they are suspended. Variations in size and shape are also introduced whenever these cells are measured away from their natural environment. Let us consider these artificial variations first.

The usual methods of examination entail one or other of the following manipulations : drying in air, dehydrating by alcohol, washing free from plasma, staining, mounting on a glass slide, etc. All of these introduce errors. For example, drying in air, by reducing the water content from about 60 per cent. to almost nothing, is bound to cause shrinking and distortion of the membrane, alteration in the pH of the colloidal matter of the corpuscle due to loss of CO_2, etc. Fixation, even by such a rapid fixative as osmic acid, produces peculiar bell-shaped forms. Nevertheless, drying, fixing and staining methods are universally employed, and the results obtained by them appear in most text-books as standard. The best that one can say of methods which take such liberties with a delicate colloidal structure is that they are rapid, and if applied in a standardised way, that they will give *comparative* results.

The cell and its environment, the erythrocyte and its plasma, must be considered together. Measured in this way, at a constant tension of CO_2, the human red cells are $8{\cdot}8\mu$ in mean diameter as

against $7{\cdot}1$–$8{\cdot}3\mu$ in dried films and $6{\cdot}8$–$7{\cdot}5$ in dried *and stained* specimens. Their greatest thickness is very nearly $0{\cdot}3$ of the diameter, *i.e.* in man, from $2{\cdot}4\mu$ in a dried specimen to $2{\cdot}7\mu$ for a natural cell. It will be seen, therefore, that if the shape is the same in both cases, the cell as it occurs in the blood stream is larger in every way than when dried in a film. The thickness across the narrowest part is about 2μ.

The volume and surface area of the cell can be calculated from the diameter and thickness. This gives the human red cell a volume of between 70 and $80\mu^3$ and an area of $120\mu^2$. The value thus obtained for the volume agrees reasonably with that found from dividing the percentage volume of cells found by the haematocrite by the number of cells actually counted per unit volume.

The size of the red cell is markedly altered by the pH of the plasma in which it is suspended. Increase of CO_2, by decreasing the alkalinity of the blood, produces swelling (cf. swelling of colloids, Chap. VIII.). Therefore venous blood will always have larger erythrocytes than arterial blood. We shall discuss the significance of this in the next chapter.

Shape. In contact with plasma the human red cell is a circular biconcave discoid. A peculiar shape like this gives one the impression of the application of a constant distorting force. If the corpuscle, as is the almost general opinion to-day, is an elastic bag filled with fluid containing, it is said, colloidal matter dispersed through it, one would expect it to be spherical. Several views have been advanced to explain the central cavities.

1. Those who consider that the cell is a sponge-like body easily find an explanation of the persistence of the shape, *i.e.* it is imposed on the cell by the stroma within it. The flattening they attribute to the loss of the nucleus and the consequent loss of some water. Nucleated red cells have about 70–90 per cent. water, while non-nucleated cells have only between 60 and 70 per cent. by volume. Unfortunately the change from the practically spherical erythroblast to the typical disc-like erythrocyte is not synchronous with the disappearance of the nucleus from the former. Further, the nucleus is not thrown out of the cell. It undergoes disintegration *in situ*, and its fragments are gradually absorbed by the cell. Flattening occurs later.

2. The shape may be due to surface forces. Since the membrane of the cell is mainly lipide, the cell, as a whole, may be regarded as a lipide drop. Shape is imposed on any such drop by surface forces. If these forces are uniformly applied, a spherical form results ; if unevenly, an egg-shaped body, and so on. It is difficult

to conceive how differences of surface tension could be produced at different parts of a cell surface bounding two fluids such as cell contents and blood plasma.

3. Ponder's view is that the erythrocyte contains fluid or semi-fluid material, and if the volume of the cell be increased by the passage of fluid into it, the diameter (equatorial axis) must diminish at the same time as the polar axis increases, until the ratio of the axis is about 1 to 1·6. Thereafter both axes increase. This investigator has worked out his theory mathematically from the principles governing the stretching of elastic membranes of spheroidal form and has constructed models which when distended assume a discoid form.

Experiments by Gough and by Secker may be considered as supporting this theory. The former's experiments also give a clear indication of the intimate relationship between the red cell and the blood plasma. On removing the last traces of plasma and suspending the red cells in isotonic saline, they assume the spherical form. The addition of serum, however, causes them to reassume their discoid form. The volume of the cell in both forms is the same. Secker showed that *unwashed* corpuscles retained the discoid form in various saline solutions, but if to any of the solutions a small quantity of insulin or of guanidine carbonate solution were added, the corpuscle became spherical. If the saline were isotonic or hypertonic the sphere had a smaller diameter than the disc. On the other hand, when a hypotonic saline was used, although the sphere was of the same diameter as before, the haemoglobin of these corpuscles gradually diffused out without apparent rupture of the plasma membrane, leaving spherical "ghosts."

Now we must admit that, at all times, as long as the cell membrane is intact, the contents of the cell must be in hydrostatic equilibrium with the plasma. That is, the forces applied to the cell membrane from without the cell must be exactly balanced by the forces applied to the membrane from within the cell. The forces tending to compress the cell are (i.) the elastic pressure of the membrane; (ii.) the osmotic pressure of the plasma colloids and of those crystalloids to which the membrane is impermeable. On the other hand, the opposing forces are the osmotic pressures of the cell colloids, viz. haemoglobin and cell-globulin β, and possibly of some crystalloids like potassium. If this balance is altered the cell will alter in size but not necessarily in shape. An increased power of imbibition conferred on the cell colloids by increased CO_2 tension, for instance, leads to a swelling of the corpuscle but no alteration in shape. Disturbance, however, of

the orientated surface layers, by removal of plasma or by disturbance of the K/Ca ratio, leads at once to altered permeability and an alteration in shape. From such experiments one infers that the corpuscular capsule in contact with plasma is not of uniform elasticity. This lack of uniformity is abolished when the cell is completely removed from plasma or serum.

The conclusion at present drawn from experiments mentioned in this chapter, viz. conductivity of whole blood compared with that of laked blood, haemolysis in general, and, finally, the colligative properties of blood and plasma about to be considered, is that the red corpuscle is a bag containing fluid and no spongework or stroma. In other words, the continuous phase of the corpuscular contents is water + crystalloids + cell globulin β, while the disperse phase is haemoglobin with some " bound " water and possibly adsorbed crystalloids. The blood as a whole, then, consists of a water-in-colloid complex in which is suspended small encapsulated drops of a colloid-in-water complex. These two systems may be compared in their properties to a series of little gelatin + collodion bags filled with phenol-dispersed-in-water, suspended in a fluid, water-in-phenol.

Contents of erythrocyte. The nature and volume of the disperse phase in the contents of the corpuscle is of considerable academic interest. The next chapter will be devoted almost entirely to the study of the function of the haemoglobin, which is the main constituent of the disperse phase within the cell. It is a conjugated protein composed of about 94 per cent. of a colourless protein—*globin*—belonging to the histone group, and 6 per cent. of a brownish ferro-porphyrin called *haem*. Haem is completely insoluble in water at a *p*H of about 7 and less, *i.e.* in neutral and acid aqueous solutions, but dissolves in the presence of alkali. Such a solution gives a diffuse absorption spectrum in the yellow region not at all like that characteristic of haemoglobin. On combining with *globin*, however, the spectrum of *reduced alkaline haematin* (haemochromogen) is obtainable from the compound. Probably four haemochromogen molecules (mol. wt. about 17,000) polymerise under the influence of an increase in the H^+ ion concentration to form one *haemoglobin* molecule (mol. wt. about 68,000). The pigment is dispersed in gel form through a sol within the membrane of the corpuscle. Actually about 32 grams of Hb and 63 grams of water are found in every 100 grams of red cells, while only 18 grams of Hb can be dissolved in 100 grams of water.

Various estimates have been made of the volume of the dispersed phase relative to the volume of the continuous phase. A know-

ledge of this is necessary if one is to explain the partition of diffusible non-electrolytes between the disperse phases of plasma and corpuscle which have been found by experiment. For example, if it were found that the partition of urea between corpuscle and plasma corresponded to the relative magnitudes of the continuous phase in those two bodies, then the conclusion could be drawn that the urea was in solution only in the dispersion media. On the other hand, if one found that the ratio of concentration of a substance in corpuscle to its concentration in plasma were greater or less than the ratio between the volumes of the continuous phases in corpuscle and plasma, then some of the substance must be adsorbed to or soluble in the disperse phase of one of the systems. Various values have been obtained for the volume of the disperse phase in the corpuscle, ranging from 33 to 65 per cent. The reason for this wide variation is to be sought in the methods employed in the investigation. The larger values are obtained when a haematocrite (*q.v.*) or other apparatus depending on centrifugal force is used. If sedimentation is not complete, and it very seldom is, high values are obtained for the volume of the red cells. Ege's method is simple and ingenious, and depends on the determination of differences in the depression of the freezing point (*q.v.*) of solutions of non-electrolyte in equal volumes of water and of red corpuscle press-juice. For example,

if the Δ of 8 grams of cane sugar in water $= 1{\cdot}22°$ C.
and of 8 grams of cane sugar in press-juice $= 1{\cdot}81°$ C.

(allowance having been made for the Δ of the press-juice alone) then the volume (*D.P.v.*) of the disperse phase of the corpuscular juice would be found from the equation:

$$(100 - D.P.v.) \times 1{\cdot}81 = 100 \times 1{\cdot}22$$

$$100 - D.P.v. = \frac{122}{1{\cdot}81} = 67 = \text{continuous phase}$$

$$\therefore \text{ volume of disperse phase} = 33 \text{ per cent.}$$

It is inferred that the dispersed phase consists almost wholly of water in haemoglobin in a continuous phase of cell-globulin β in water, both phases being enclosed in a membrane composed of protein, lecithin and other phosphatides, cholesterol, etc.

Haemolysis.

As the haemoglobin is held in the corpuscle in a state in which it is more concentrated than it could possibly be when in solution, the process of putting it into solution ought to alter some of the physical characters of blood. Blood diluted with an isotonic

solution, so that the corpuscle is not subjected to osmotic strain, appears yellow and somewhat opaque. If now, the capsules of the corpuscles are damaged so that the Hb is set free and goes into solution in the aqueous saline solution, the liquid will become translucent and a deep red in colour. This is known as haemolysis, and haemolysed blood, because it is similar in colour to crimson-lac-resin (a gum extruded from tropical trees after puncture by the lac insect), is said to be laked.

Blood may be laked by various methods :

1. *Mechanical*, grinding corpuscles with sand or powdered glass and taking up with salt solution.

2. *Physical*, freezing and thawing, heat, condenser discharges, or similar methods.

3. *Endosmosis*, dropping blood into water or into a hypo-tonic solution.

4. *Exosmosis* (see crenation), dropping blood into a hypertonic solution.

5. *Action on Lipoid constituents of capsule.*
   i. Anaesthetics are lipoid solvents.
   ii. Bile salts and pigments affect permeability of lipoid membranes.
   iii. Glucosides, *e.g.* saponin, are adsorbed by the membrane (Willard Gibbs' Law), because they lower surface tension. They then insert themselves into the texture of the membrane and increase its permeability (see p. 138).

6. *Biological Agents.*
   i. Toxins of certain bacteria produce haemolysis, *e.g.* tetanolysin of B. Tetani ; megatheris lysin of B. Megatherium ; toxic lysins of staphylococcus and B. pyocyaneus.
   ii. Cobra and rattlesnake venom cause laking both *in vivo* and *in vitro.*
   iii. The serum of one animal is often haemolytic for the blood of a different species.
   iv. Certain phyto-albumins, *e.g.* abrin, ricin, and robin, produce haemolysis.

7. *Chemical Agents.*

Haemolysis may be caused by the ingestion or injection of certain drugs, *e.g.* chlorates, nitrites, nitrobenzene, aniline derivatives (*e.g.* acetanilide and phenacetin), quinine. These, generally, partially convert the haemoglobin into methaemoglobin (*q.v.*).

The laking of blood thus depends on altering the permeability of the capsule of the erythrocyte. Normally this membrane is

impermeable to colloids and to most crystalloids. This has been determined by estimating the electrical conductivity of blood before and after laking. The corpuscles hinder the passage of small electrical charges because their walls are impermeable to ions carrying the charge. On rupturing the membranes these ions get a free passage and the conductivity of the blood increases. That this increase is not due to the liberation of haemoglobin may be shown by fixing the corpuscles with formalin, which prevents the egress of the pigment but not of the salts.

Laked blood has a lower viscosity than whole blood due to the lack of the pseudo-viscosity caused by the corpuscles.

Haemolysis by freezing and thawing.

It has been noticed that blood could be repeatedly frozen and thawed as in the determination of the osmotic pressure (freezing point method) with little or no laking. If, however, the blood was suddenly cooled to below the freezing point of water, was kept at that temperature for a long time, or was rapidly thawed, pronounced haemolysis was produced. Burton-Opitz prepared completely laked blood by eight times freezing it solid and thawing it rapidly. The mechanism of this laking is not clearly understood. Possibly the withdrawal of water from the membrane to form ice might be adduced as sufficient reason (Guthrie). (Cf. Test for frozen meat.)

Endosmotic laking.

Normally, the corpuscle has within it a concentration of colloids and of crystalloids isotonic with 0·9 per cent. sodium chloride. A similar state prevails, as we have seen, in the plasma in which the corpuscle is immersed. The corpuscular membrane is almost semipermeable; that is, water may pass through it, but not certain salts in solution and not colloids. If the concentration of salts and colloids inside and out of the membrane were not exactly balanced, water would pass from the place of low concentration to that of high concentration (see Osmotic pressure, Chap. V.). That means that blood dropped into water or into a solution of lower concentration than 0·9 NaCl would gain water. Water would pass into the corpuscle, cause it to swell, and when the limit of elasticity had been passed, the corpuscle would burst and scatter its contents into the fluid.

Crenation.

Loss of water by evaporation or by immersion in a solution more concentrated than 0·9 per cent. NaCl causes the corpuscles to

shrink and shrivel. They then break up into fragments, due to inequalities in the tensile strength of the corpuscle. Most peculiarly the first stage in exosmotic laking is a swelling of the corpuscle. Some change in the physico-chemical state of the protein moiety in the envelope is indicated. It has been shown that the power of colloids to imbibe water may be altered by alterations in their crystalloid content.

Colligative properties of whole blood.

The suspension of small bodies like blood corpuscles in plasma should not materially affect the values of vapour pressure, osmotic pressure, etc. This is found to be the case. Using average values taken from Grollman's paper referred to above, we find that blood has practically the same vapour pressure and osmotic pressure as its plasma at body temperature. Plasma is, however, slightly richer in free salts, as shown by a slightly greater depression of the freezing point. The difference is so slight that one might hesitate, in view of the unstable nature of the bound salts of "separated" plasma, to accept it as significant.

TABLE XLII

COLLIGATIVE PROPERTIES OF BLOOD AND "SEPARATED" PLASMA AT 37·5° C. (DOG)

	Vapour Pressure. mm. Hg.	Depression of Freezing Point. °C.	Osmotic Pressure. Atmospheres.*
Blood	48·08	0·602	8·8
	48·12	0·585	7·6
	48·09	0·590	8·5
Plasma	48·09	0·616	8·5
	48·12	0·605	7·6
	48·11	0·604	7·9
	48·08	0·620	8·8

* Calculated from vapour pressures. Grollman: *Jour. Gen. Physiol.*, XI., 1928.

Viscosity of whole blood.

The presence of corpuscles prolongs the time taken by whole blood to traverse a viscosimeter as compared with plasma. The following figures show this. Serum was used instead of plasma, to prevent complications by clot formation.

TABLE XLIII

		Viscosity at 32° C.
Serum +	$\times 0$ Corpuscles	1·9
,,	$+ 3{\cdot}2 \times 10^6$,,	3·3
,,	$+ 6{\cdot}3 \times 10^6$,,	4·9
,,	$+ 12{\cdot}6 \times 10^6$,,	15·6

The last high value was due to the mechanical blocking of the capillary tube by the corpuscles which tend to agglutinate when so concentrated.

The capsule and its contents are colloidal in character. Acids increase the power of colloids to imbibe water, and, therefore, one would expect that CO_2 would cause an increased imbibition of water by the corpuscles and, consequently, increase the viscosity of blood, due (*a*) principally to the absorption of water from the plasma rendering it more viscous and (*b*) the swelling of the corpuscle itself. The experimental proof of this has not been very satisfactory, but some workers have observed increased viscosity in venous blood, especially in cases where the unsaturation of haemoglobin is low (pneumonia, gas poisoning).

Viscosimetric measurements afford another means of determining the volume of blood corpuscles. Viscosity depends principally on the total volume of corpuscles per unit volume of fluid. Having determined (i.) the viscosity of whole blood $= pc$, and (ii.) that of the plasma $= p$, the total corpuscular volume K may be derived from the formula

$$1 - K = \frac{p}{pc}.$$

If the total number of corpuscles per unit volume be N, then the average volume of each will be K/N.

The results obtained from such an indirect method are fairly regular, but cannot be considered as absolutely accurate, as viscosity does not depend, in principle, on either the number or the volume of corpuscles, but on the *effective surface*, *i.e.* on the area liable to friction (see relation between viscosity and blood-pressure).

FURTHER READING

PONDER. "The Erythrocyte and the Action of Simple Haemolysins." Oliver and Boyd.

CHAPTER XXIII

RESPIRATORY FUNCTION OF THE BLOOD

"There is no instance in which it can be proved that an organ increases its activity under physiological conditions without also increasing its demand for oxygen."
BARCROFT.

THE erythrocyte assumes importance as the carrier of the respiratory gases, oxygen and carbon-dioxide. Air has an average composition of about 79 volumes per cent. of nitrogen and 21 of oxygen. The amount of carbon-dioxide present is so small (0·03 per cent.) that it may for the present be neglected. The partial pressure of oxygen, therefore, at normal pressure would be $\frac{21}{100} \times 760 = 159{\cdot}6$ mm. of mercury. The partial pressure of oxygen in the lung is, on account of the carbon-dioxide and aqueous vapour present, much less than this. Alveolar air contains in 100 c.c. about 5·5 vols. of CO_2, 13 vols. of O_2 and 79·5 vols. of N. Their partial pressures will be (at normal barometric pressure)

$$\begin{aligned} O_2 &= \tfrac{13}{100} \times 760 = 98{\cdot}8 \text{ mm. Hg.} \\ CO_2 &= \tfrac{5{\cdot}5}{100} \times 760 = 41{\cdot}8 \text{ mm. Hg.} \\ N &= \tfrac{79{\cdot}5}{100} \times 760 = 604{\cdot}2 \text{ mm. Hg.} \end{aligned}$$

The partial pressure of the oxygen in the lung is thus about 2/3 of its partial pressure in the atmosphere. The percentage of nitrogen shows an apparent increase because the total air is decreased in the ratio $\frac{390}{350}$ by the absorption of oxygen without a corresponding production of carbon-dioxide. Then, too, the tension of aqueous vapour at body temperature is by no means negligible. It amounts to about 50 mm. of Hg. That is, dry air at normal temperature and pressure when taken into the body has its pressure reduced to $760 - 50 = 710$ mm. Hg. This causes the actual oxygen pressure to fall to 92·3, and carbon-dioxide to 39 mm. Hg.

The quantity of gas by weight (or by volume reduced to N.T.P.) dissolved in a liquid is proportional to its partial pressure provided chemical and physical conditions remain constant. If, for instance, the pressure of the gas be doubled, twice as much of it will go into solution. The appended table contains experimental verifications of this **Law of Henry.**

TABLE XLIV

SOLUBILITY OF CO_2 IN WATER AT 15° C.

P.	V.	V./P.
69·8	0·94	0·0135
128·9	1·86	0·0144
200·2	2·90	0·0145
311·0	4·5	0·0145

where P = pressure in mm. of Hg of CO_2,
V = volume of CO_2 (measured at N.T.P.) absorbed by 1 c.c. of water at 15° C.

(The same *volume* of gas at constant temperature is absorbed by the fluid, no matter what the pressure is. Increase of pressure proportionately increases the weight of unit volume. Thus, if 1 volume of water dissolves 1 volume of gas weighing 1 gram at 1 atmosphere pressure, then, if the pressure be raised to 2 atmospheres, 1 volume of water would dissolve 1 volume of the gas weighing 2 grams, or if reduced to normal pressure, 1 volume of water would dissolve 2 volumes of the gas weighing 2 grams.)

Absorption coefficient (usually denoted by the Greek letter α).

Different gases, just like different solids, vary in their solubilities. The volume of gas (at N.T.P.) which dissolves in 1 c.c. of water under a pressure of 1 atmosphere is termed its absorption coefficient, *e.g.* 1 c.c. of water will dissolve at N.T.P. 0·0489 c.c. of oxygen, 0·0239 c.c. of nitrogen, and 1·713 c.c. of carbon-dioxide. The volume of gas absorbed by 1 c.c. of water under any pressure may be found by the following equation :

$$L = \alpha p,$$

where L = amount of gas dissolved,
α = absorption coefficient,
p = pressure in atmospheres.

Variations in temperature alter the amount of gas a fluid may take up.

The amount of gas absorbed by a fluid decreases as the temperature of the fluid (and gas) is increased and *vice versâ.*

TABLE XLV

ABSORPTION COEFFICIENTS AT VARIOUS TEMPERATURES

Temperature.	Oxygen.	Nitrogen.	Carbon-dioxide.
0° C.	0·0489	0·0239	1·713
10° C.	0·0380	0·0196	1·194
20° C.	0·0310	0·0164	0·878
30° C.	0·0262	0·0138	0·665
40° C.	0·0231	0·0118	0·530

Effect of solutes.

Plasma, as we have seen, is a hydrophilic colloid in which a large amount of water is dispersed through a hydrated protein-crystalloid

complex; that is, only a certain amount of the water present (the true disperse phase) is chemically free. The large bulk of the fluid present is firmly bound and closely packed in orientated layers to the colloid, or serves as water of hydration for the crystalloidal components. The colloidally bound water is considered by R. A. Gortner to be capable of " dissolving " more gas than free water. On the other hand, many experiments in which various solutes were added to plasma tend to show that the presence of free crystalloids decreases the amount of gas that it is able to hold.

Gas-holding power of plasma.

It has been found that at the pressure of about 90 mm. Hg, which we saw oxygen had in the lung, 100 c.c. of plasma will dissolve 0·273 c.c. of oxygen (measured at N.T.P.). If we consider that the tension of oxygen in the tissues cannot be less than zero, and one has as a maximum amount 0·273 c.c. of oxygen for every 100 c.c. of plasma passing through the tissue, a cat's gastrocnemius muscle weighing 20 grams and using about 0·24 c.c. of oxygen per minute would, therefore, need to have *at least* 100 c.c. of plasma passing through it per minute. A warm-blooded animal would need to have about twice as much plasma by volume as the present volume of its body. The body would be unable to cope with the weight of its own circulating fluid. For example, the average man weighing 66 kg. would have to carry, *in addition*, at least 140 kg. of plasma, thus increasing his total weight to 206 kilos. As Barcroft puts it, " man would never have attained any activity which the lobster does not possess, or had he done so it would have been with a body as minute as the fly's." In the experiment quoted above the actual amount of *blood* passing through the cat's muscle was 4·5 c.c. per minute—just under a twentieth of the amount necessary when plasma alone was considered. This is due to the specific oxygen capacity of the haemoglobin in the blood.

The following table gives the volume (in c.c. at N.T.P.) of oxygen, nitrogen and carbon-dioxide which will dissolve in 100 c.c. of fluid at 38° C. and 760 mm. pressure.

TABLE XLVI

	Oxygen.	Nitrogen.	Carbon-dioxide.
Water	2·37	1·2	55·5
" Separated " plasma .	2·3	1·2	54·1
" True " plasma . .	2·2	1·1	51·1

In addition to the amount *dissolved* in " true " plasma one has to consider the amount *held* by the haemoglobin. The table given

below contains the results of a series of experiments on the blood of a horse, where the amount of oxygen in the blood was determined at various pressures.

TABLE XLVII

Oxygen tension in mm. of Hg. (1)	Oxygen in c.c. (at N.T.P.) per 100 c.c. of blood. Bound by Haemoglobin. (2)	Dissolved in Plasma. (3)	Degree of Saturation of Hb. %. (4)	Degree of Dissociation % (unsaturation). [100-(4)]
10	6·0	0·020	30·0	70·0
20	12·9	0·041	64·7	35·3
30	16·3	0·061	81·6	18·4
40	18·1	0·081	90·4	9·6
50	19·1	0·101	95·4	4·6
60	19·5	0·121	97·6	2·4
70	19·8	0·141	98·8	1·2
80	19·9	0·162	99·5	0·5
90	**19·95**	**0·182**	**99·8**	**0·2**
150	20	0·303	100	0·0

From the figures in Table XLVII. it may be seen that much the greater proportion of the oxygen is carried in combination with the haemoglobin of the cells, and a relatively negligible proportion in solution in the plasma.

Oxygen load of corpuscle.

The average normal man has about 5 litres of blood; 2·5 litres of this is occupied by 25×10^{12} red corpuscles with a total surface of about 3,000 sq. metres. When fully saturated with oxygen at atmospheric pressure (160 mm. Hg) each litre of blood will take up 200 c.c. of oxygen. Leaving out of account the relatively small amounts of oxygen carried in solution in the plasma and adsorbed by the plasma proteins, we may calculate that each corpuscle will carry oxygen to the extent of

$$\frac{\text{Total oxygen capacity of blood}}{\text{Total number of corpuscles}} = \frac{1000}{25 \times 10^{12}} = 4 \times 10^{-11} \text{ c.c.}$$

Each cubic centimetre of oxygen will need 25×10^{9} corpuscles to carry it.

Further, the total *iron* content of human blood is about 2·5 grams (Schmidt).

That is, each gram of iron is associated with 1000/2·5 = 400 c.c. of oxygen—a figure *closely* approximating to Barcroft's experi-

mental finding of 401 c.c. Normal blood contains 14 grams per cent. of haemoglobin. It follows that each corpuscle carries $\frac{140 \times 5}{25 \times 10^{12}} = 28 \times 10^{-12}$ grams of haemoglobin. In other words, each cubic centimetre of oxygen postulates the presence of

$$\frac{28 \times 25 \times 10^{9}}{10^{12}} = 0{\cdot}7 \text{ gram of Hb.}$$

The iron content of Hb must therefore be $\frac{10}{7 \times 400} = \frac{1}{280}$ gram per gram of Hb. Putting this in molecular terms, one molecular proportion of iron (56 grams) enters into the composition of 15,680 grams of Hb. The molecular weight generally given for Hb is 16,660.

Nature of union between oxygen and haemoglobin.

Evidence has been produced which shows that when haemoglobin is fully saturated with oxygen there are 401 c.c. of oxygen for every gram of iron. That is, each combining proportion of iron (56 grams) co-exists with two combining proportions (32 grams) of oxygen. From this, some deduce the presence of a compound FeO_2 in haemoglobin. That may be. On the other hand, the idea of a chemical combination between haemoglobin and oxygen raises hosts of difficulties which would not arise on the hypothesis of adsorption of gas on a colloidal surface. However, no reliable direct experimental evidence bearing on this has come to hand. If analogies could be taken as a proof instead of analysis, the adsorption theory would hold the field undisputed.

Conditions controlling the reaction between haemoglobin and oxygen.

Whatever may be the nature of the union between oxygen and haemoglobin, it is established that the reaction between them is a reversible one, the equilibrium point being determined by the tension of oxygen and the active mass of haemoglobin, when all other conditions, such as temperature, hydrogen ion concentration, etc., are kept constant.

In Table XLVII., if the tensions (1) are taken as abscissae, and the degree of saturation (4) as ordinates, a curve (Fig. 82, heavy line) is obtained, called *the dissociation curve of blood.* It has been shown by Hill that this curve can be approximately represented by the equation

$$K = \frac{[Hb]\,[O_2]^n}{[HbO_2]}$$

The dissociation curve for pure haemoglobin is also found in Fig. 82, dotted line. It differs from that of whole blood in that its contour is that of a rectangular hyperbole, which can be represented approximately by the equation

$$K = \frac{[Hb] \times [O_2]}{[HbO_2]}$$

(Henderson)

Influence of the solutes of blood on the dissociation curve.

From Fig. 82 it may be seen that, at all oxygen tensions, the

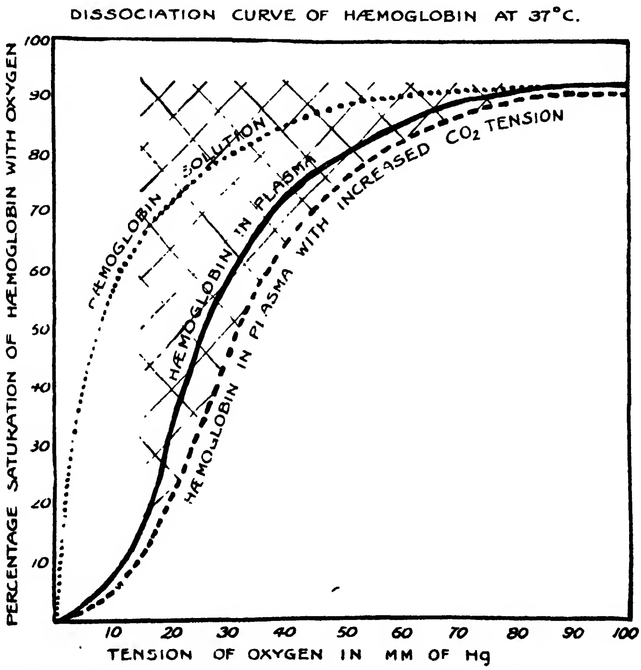


FIG. 82.—Dissociation curve representing the equilibrium between oxygen tension, oxy-haemoglobin and reduced haemoglobin :

.... — Curve from pure 14% haemoglobin solution.
———————— — ,, ,, 14% haemoglobin in plasma.
– – – – – – – – — ,, ,, ,, ,, ,, with increased tension of carbon-dioxide.

The hatched portion represents the amount of haemoglobin which gives up its oxygen when the oxygen tension is reduced from 100 to 15 mm. Hg.

percentage saturation of haemoglobin with oxygen is less in blood than in a solution of pure haemoglobin, *although the difference is small at tensions above about* 80 *mm. of Hg.*

The dissociation curves of haemoglobin in solutions of sodium chloride of approximately the same osmotic concentration as plasma (about 0·9 per cent.) (*q.v.*) are found to lie nearer the

dissociation curve of blood, but to retain the same general shape as the curve for pure haemoglobin.

Influence of H^+ concentration.

An increase, however, in the carbon-dioxide tension of the haemoglobin solution produces a curve of the sigmoid shape, typical of the blood dissociation curve, and, *in fact, the dissociation curve of haemoglobin in a solution containing the solutes of blood, including CO_2, in the proportion in which they occur in blood, lies point for point on the dissociation curve of that blood.* The presence of any other acid in the blood, tending to increase the H^+ concentration, as will be shown later, liberates CO_2 from bicarbonates present, and, therefore, has the same effect on the oxygen dissociation curve as an equivalent increase in CO_2 tension. It might be thought that this influence of carbon-dioxide on the union between haemoglobin and oxygen was a direct one, the oxygen in the oxy-haemoglobin being simply replaced by carbon-dioxide, but there is no molecular equivalence between the amounts of the two gases involved in the reaction, and, therefore, this simple replacement cannot be the explanation of the influence.

Examination of the curves of Fig. 82 reveals the importance to the body of the part played by the solutes of the blood in the transport of oxygen. At high oxygen tensions, the presence of salts and CO_2 in the blood does not materially decrease the percentage saturation of the haemoglobin, but at low oxygen tensions, such as are found in the tissues, it enables much more oxygen to be given off by the blood than would be the case in a solution of pure haemoglobin.

In the tissues the tension of oxygen is low, say 15 mm. Hg. At this tension whole blood can only be 15 per cent. saturated. Therefore the oxygen carried by 77 per cent. (92 — 15) of the haemoglobin is discharged (where the blood in the lungs is supposed to be 92 per cent. saturated). On the other hand, pure haemoglobin is still 65 per cent. saturated at 15 mm. Hg. It will only be able to discharge the oxygen borne by 27 per cent. of its haemoglobin. In other words, because of the presence of solutes, whole blood is able to set free in the tissues the full amount of oxygen that could be obtained from pure haemoglobin, with, *in addition*, the amount that would be carried by the haemoglobin represented by the space between the heavy curve and the dotted curve in Fig. 82. *That is, solutes so aid in the unloading of oxygen that 50 per cent. of the haemoglobin that would otherwise have retained its oxygen is induced to give it up to the tissues.* Because of the solutes, whole blood becomes an effective carrier of oxygen, and the total volume of fluid (and mass of haemoglobin) is kept within reasonable limits.

The effect of the carbon-dioxide tension is particularly important at very low oxygen tensions, as with increasing carbon-dioxide

tension the percentage saturation of haemoglobin with oxygen at these low oxygen tensions becomes very small.

In the lung, CO_2 passes from the blood into the alveolar air, and the hydrogen ion concentration of the plasma tends to decrease. At the ordinary alveolar tension of oxygen this has little effect on the combination of oxygen and haemoglobin, but where the alveolar tension of oxygen is reduced, as at high altitudes, the exchange of gases occurs under conditions in which the CO_2 tension is increasingly important, and the adequate removal of the CO_2, and consequent decrease of H^+ concentration in the plasma, is an important factor in permitting the picking up of an adequate load of oxygen by the haemoglobin.

From curve 82 it may be seen that, if the exchange of gases were to take place in the lungs at an oxygen tension of 50 mm., and if the curve indicated by the broken line represented the haemoglobin dissociation curve at the CO_2 tension of venous blood, and the thick line represented the same curve at the CO_2 tension of the alveolar air, then the mere passage of the CO_2 from the blood into the alveolar air would enable the haemoglobin to take up more oxygen. *Thus the action of the CO_2 on the reaction between haemoglobin and oxygen is such that the taking up of oxygen in the lungs and the unloading of it in the tissues are both facilitated.* So it appears that the CO_2 tension of the blood is an important factor in defining the oxygen dissociation curve, and that the constant K in Hill's equation must be a function of the carbon-dioxide tension.

It has been found that Hill's equation may be written

$$\frac{H_2CO_3 + 7{\cdot}7}{0{\cdot}014} = \frac{[Hb]\,[O_2]^n}{[HbO_2]},$$

where $$K = \frac{[H_2CO_3] + 7{\cdot}7}{0{\cdot}014}.$$

How the tissues unload the oxygen.

(1) **Distortion.** The corpuscle is considered as a fluid drop with an outer coat of orientated lipoid matter. The colloidal haemoglobin is dispersed through the liquid contents. These little barges, $8{\cdot}8\mu$ in diameter (p. 313), squeeze along the capillary vessels in the tissues. During their passage along a tube with a diameter less than their own the corpuscles naturally undergo distortion. This distortion has at least one effect on the loading and unloading of the oxygen from the corpuscles. It puts on the brake, slows down the corpuscles and gives the dock-labourers and others opportunity to carry out their work.

(2) Another physical factor comes into play, viz., **alterations of temperature,** and that has a profound effect on both the amount of gas liberated and the speed at which it is handled. The temperature in the lung where oxygen is taken on board is usually less than 37° C., while the temperature of active tissue may be greater (see Chap. XXXII.). Increase in temperature increases the desaturation of haemoglobin. The amount of desaturation brought about by an increase in temperature may be calculated from the laws of van't Hoff and Arrhenius. The process of saturation and desaturation may be represented by the reaction formula

$$Hb + O_2 \rightleftharpoons HbO_2.$$

The velocity of this reaction depends, other things being equal, on the active masses of oxygen, C_0, and of haemoglobin, C_R,

$$i.e.\ v = k\,(C_0 \times C_R).$$

Now k_1, the velocity constant of the saturation process, and k_2, the corresponding constant for desaturation, vary with the temperature. We have seen (p. 323) that a, the absorption coefficient of oxygen in blood, varies inversely with the temperature.

$$C_H = C_R \times \left(\frac{k_1}{k_2} \times \frac{a}{760}\right) \times p = C_R \cdot K \cdot p,$$

where C_H = concentration of oxyhaemoglobin and p = oxygen pressure.

This value, K, is constant for each temperature, and by the law of van't Hoff the values of K for any two temperatures T_1 and T_2 are related by the equation

$$K_2 = K_1 \epsilon^{\frac{-q}{2} \cdot \frac{T_2 - T_1}{T_2 T_1}}$$

(ϵ = base of Napierian logs, q = heat evolved when 1 gram molecule of Hb unites with 1 gram molecule of oxygen).

For example, let us try to determine what desaturation would arise from raising the temperature from 36° C. to 39° C.

Here $\quad T_1 = 273 + 36 = 309$ absolute,
$\qquad\quad T_2 = 273 + 39 = 312$,,
and $\qquad q = 28{,}000$ cals.,

$$K_{39} = K_{36}\,\epsilon^{\frac{-28000}{2} \cdot \frac{3}{309 \times 312}} = K_{36}\,\epsilon^{-0{\cdot}4356}.$$

If K_{36} be 30 per cent., then K_{39} is equal to $30\epsilon^{-0{\cdot}4356} = 19{\cdot}4$, we find that haemoglobin which was 30 per cent. saturated at 36° becomes only 19·4 per cent. saturated at 39°.

An increase in 3° C. between the values of 36 and 39 causes the HbO_2 to lose oxygen to the extent of about 10 per cent. of full saturation.

(3) **Carbon-dioxide.** A physico-chemical factor, however, is much more potent than temperature in producing desaturation. Active tissues tend to become acid. In dealing with muscle, we have seen how lactic acid is set free as the result of activity and how oxygen is required before it can be replaced in the muscle complex. This free lactic acid performs another service. Either *directly* by partially diffusing into the surrounding lymph or *indirectly* by producing alterations in the Helmholtz (polarising) electric charge, it causes a potential alteration in the hydrogen ion concentration of the tissue fluid. By a series of changes which we have already briefly considered, and to which we shall return, the net effect is to increase the tension of CO_2 in the capillaries. The molecules of CO_2 are to be the new passengers on the erythrocytes, and because of their acidity they cause an aggregation of the colloidal particles of haemoglobin and, as has already been indicated, a marked desaturation. Carbon-dioxide acts as if the tension of oxygen in the tissues were reduced to 10/24ths of its real value. *That is, haemoglobin parts with as much oxygen at a tissue tension of oxygen of 24 mm. Hg. as if the tension of oxygen were only 10 mm.* For example, blood which in the absence of CO_2 would be 30 per cent. desaturated is actually desaturated to the extent of 78 per cent. by the presence of a CO_2 tension of 40 mm. Hg. The blood becomes now a carrier of CO_2 from the tissues to the lungs (Fig. 82, dash line curve).

Handling of Oxygen. Consider an inland village supplied by a canal coming as close as possible to the community. Internal communication is effected by waterways fed from the canal. The by-products of manufacture (sent elsewhere for elaboration) are transported along these waterways to the canal and the same gangs of labourers unload the raw material from the barges and float it up to the factories.

To take a specific instance, muscle, as a result of its activity, produces carbon-dioxide. This weak acid acts on the sodium hydrogen phosphate of the tissue fluid according to the following equation :

$$H_2CO_3 + Na_2HPO_4 \rightleftharpoons NaH_2PO_4 + NaHCO_3.$$

The sodium bicarbonate so formed finds its way into the blood stream, where it is in equilibrium with free dissolved carbon-dioxide, so that the volume of CO_2 in solution is 1/20 of the volume of combined CO_2.

The result of the influx of $NaHCO_3$ is to increase the volume of

free CO_2 in order to preserve this ratio $\frac{CO_2 \text{ free}}{CO_2 \text{ combined}} = \frac{1}{20}$. That is, the CO_2 tension in the muscle capillaries tends to increase, and increase of CO_2 tension causes increased unloading of oxygen from oxy-haemoglobin.

Increased activity postulates increased energy usage, which renders necessary an immediately increased supply of oxygen. The amount of oxygen required is liberated by the desaturating action of CO_2—the main chemical product of the activity.

The amount of oxygen in the blood does not control oxidation in the tissues, but the call for oxygen by the tissues controls the rate of unloading of oxygen.

Transport of carbon-dioxide.

The principle underlying the transport of carbon-dioxide is identical with that enunciated for oxygen. In the tissues the tension of carbon-dioxide is relatively high and the gas passes to the blood, is carried to the lungs, and is there eliminated. The erythrocyte, once freed from its load of oxygen, takes on a cargo of carbon-dioxide. Part of this cargo is carried by the haemoglobin and part is dissolved in the corpuscular lipoid envelope causing it to swell. Lipoid is capable of dissolving very large amounts of CO_2. But the erythrocytes are not the sole means of transport. Carbon-dioxide is about twenty-five times as soluble in water as oxygen under similar conditions. Relatively more CO_2 will, therefore, be carried in true solution in the plasma. *In addition* to this amount (which we have just seen is carefully regulated) a considerable quantity of the gas is adsorbed to the various colloids of the plasma. (i.) Each gram of fibrinogen can carry 1/30 gram of CO_2. (ii.) Serum proteins may adsorb a measurable quantity of carbon-dioxide—at the lowest estimate, over 5 per cent. It is obvious that while these factors may almost be neglected in the consideration of the transport of oxygen, they have to be reckoned with in the case of carbon-dioxide.

TABLE XLVIII

PARTITION OF Co_2 IN 100 C.C. OF DEFIBRINATED BLOOD (HAEMATOCRITE VALUE = 51)

	CO_2 tension.	Vol. of CO_2 in blood.	Vol. of CO_2 in serum.	Vol. of CO_2 in corpuscles.
Oxygenated .	40	45·0	25·9	19·1
Reduced .	40	50·4	28·0	22·4

That is, in arterial (whole) blood there is about 50 c.c. of CO_2, of which amount the fibrinogen carries about 5 c.c., the serum

(proteins, water and crystalloid bases) about 26 c.c., and the corpuscles about 19 c.c.

As stated above, the free carbon-dioxide of the blood represents only about 1/20 of the total carbon-dioxide carried. The forms in which the combined carbon-dioxide of the blood is carried must therefore be sought.

Transport of CO_2 in chemical combination with the blood constituents.

It is assumed that the CO_2 carried in the blood in chemical combination is found there as bicarbonates, because the whole of it may be removed if the blood is treated with an acid stronger than H_2CO_3. This latter reaction is assumed to be of the nature expressed by $BHCO_3 + HA = BA + H_2CO_3$, where B is a basic radicle and HA is an acid. It is, however, found that the whole of the CO_2 of the blood may be removed by subjecting the blood to a high vacuum. This is not the case for a simple bicarbonate solution, which under a vacuum only gives off half its CO_2 ($2BHCO_3 = B_2CO_3 + H_2O + CO_2$). Plasma subjected to a vacuum reacts like a pure solution of bicarbonate and gives off only half its CO_2. The re-addition of erythrocytes to such plasma, which is again subjected to a high vacuum, permits the removal of the remaining CO_2. There is, therefore, some substance in the red cells, which acts as a weak, non-volatile acid in its relation to the carbon dioxide of the blood, and can displace the latter from its combination as carbonates if each small amount of the latter is removed as produced, owing to the low pressure at which the reaction is carried out. *It has been found that this substance is the haemoglobin of the red blood corpuscle.*

$$BHCO_3 + \underset{\text{(weak)}}{HA} \longrightarrow BA + H_2O + CO_2^{*} \text{ (*volatile—removed as formed).}$$

Absorption curve of CO_2 in blood.

A curve showing the volume of gas absorbed by blood under different pressures of the gas can be drawn for carbon-dioxide as for oxygen. The general shape of the curve is rather like that of the absorption of oxygen by a solution of pure haemoglobin than like the oxygen absorption curve of the blood, *i.e.* it is not sigmoid. *The volume of carbon-dioxide absorbed at a given tension is found to depend on the degree of oxygenation of the haemoglobin.* The higher the saturation of the haemoglobin with oxygen, the less the CO_2 absorption. (The converse influence of CO_2 in O_2 absorption has already been noted.)

The hydrogen ion concentration is also an important factor in

determining the volume of CO_2 absorbed, the volume increasing as H^+ decreases.

It has been stated that the bound CO_2 of the blood is found as bicarbonates produced by the combination of CO_2 in solution as H_2CO_3 with bases of the blood. But the blood, either venous or arterial, has a *p*H very near the neutral point, and cannot contain any considerable quantity of free base to be neutralised by the carbonic acid. The mechanism by which base is liberated for combination with the respiratory CO_2 without any considerable change in the *p*H of the blood has been shown to be dependent on the physical and chemical characteristics of haemoglobin and its compounds. The part played by this compound in the transport of oxygen has been indicated. It is equally important in the transport of carbon-dioxide.

By virtue of its chemical composition, haemoglobin in solution can dissociate, either as an acid or as a base, according to the *p*H of the solution ; in other words, it is amphoteric (*q.v.*). Therefore, in solutions at a *p*H on the alkaline side of its isoelectric point (about *p*H 6·8), haemoglobin reacts as a weak acid and can combine with bases. In solutions of *p*H less than 6·8 (*i.e.* more acid), haemoglobin reacts as a weak base and can combine directly with carbon-dioxide. Blood under ordinary conditions *in vivo* does not reach a *p*H less than 6·8, and, therefore, the haemoglobin in it always reacts as a weak acid, and cannot combine directly with carbon-dioxide. Its action in the transport of the latter is, therefore, an indirect one.

When carbon-dioxide enters the blood it reacts with the weak salts of haemoglobin to liberate the free acid haemoglobin and form bicarbonates, *i.e.* $B.Hb + H_2CO_3 = BHCO_3 + H.HB$. Haemoglobin is a very weak acid, and exerts a very slight influence on the *p*H of the red cell contents. Oxy-haemoglobin, on the other hand, acts as though it is a much stronger acid.

It is known that where a base insufficient for the neutralisation of both is added to a mixture of two acids, the proportion of the salts formed from the two acids depends on the relative " strengths " of the acids and also upon their relative concentrations in the mixture. In the cells there appears to be a fixed quantity of base which is distributed between the acids, haemoglogin and carbon-dioxide, according to their relative strength and concentrations. The concentration of haemoglobin can only be varied by variations in total cell volume, as the cell is impermeable to haemoglobin, but the concentration of carbon-dioxide in the red cell depends on the carbon-dioxide tension of the plasma, which again is determined by the CO_2 tension in the lungs or tissues. The concentration of

carbon-dioxide in the erythrocyte is, therefore, subject to considerable variation, and will tend to decrease in the lung, since the alveolar carbon-dioxide tension is low. The " strength " of the carbon-dioxide in solution as carbonic acid is unchanged, but, as has already been indicated, oxygenation in the lung causes a marked increase in the acid strength of haemoglobin. The reaction $BHCO_3 + H.Hb \rightleftharpoons B.Hb + H_2O + CO_2$ is facilitated in the direction from left to right, both by virtue of the increased acidity of the oxyhaemoglobin, which, therefore, needs more alkali to neutralise, and also by the escape of the carbon-dioxide, which lowers the concentration of carbonic acid in the cells relative to the concentration of haemoglobin.

The quantitative importance of this redistribution of base may be appreciated from the following figures taken from Van Slyke.

At the ordinary hydrogen ion concentration of blood (pH 7·4), 1 mol. reduced haemoglobin ($\equiv$ 1 mol. O_2) combines with about 1·5 equivalents of alkali.

This same haemoglobin, *in oxygenated form*, combines with a little over 2 equivalents of alkali.

$\therefore$ the reduction of 1 molecule of oxygenated haemoglobin in the tissues sets free about 0·6 equivalents of alkali to combine with the CO_2 produced in the tissues.

If the respiratory quotient is 0·8, and 0·8 molecules of CO_2 are produced for every molecule of oxygen absorbed, then 0·6 molecules of CO_2 (*i.e.* 75 per cent. of the total) will be carried by alkali liberated from combination with oxy-haemoglobin by the reduction of the latter. The remaining 25 per cent. of the carbon-dioxide is carried by reaction with certain salts of weak acids, including H_2HbO_2, present in the blood, the reactions being of the type

$$B_2HPO_4 + H_2CO_3 = BH_2PO_4 + BHCO_3.$$

These latter reactions are accompanied by a very slight increase in hydrogen ion concentration. The weak acids involved in these reactions are mainly phosphates and the proteins of plasma and cells.

It would seem, then, that cells carry at least 80 per cent. of the respiratory carbon-dioxide of the blood (*i.e.* the carbon-dioxide picked up by the blood from the tissues, and excreted in the lungs, as distinct from that concentration of carbon-dioxide which is present alike in venous and arterial blood). Examination of arterial and venous blood from any individual under given conditions reveals, however, that the excess of carbon-dioxide carried by the venous blood over that carried by the arterial blood is distributed in approximately the ratio of 3 : 3 between serum and cells. (Table XLVIII.)

TABLE XLIX

100 c.c. of Arterial Blood (CO_2 tension 40 mm., O_2 tension 100 mm.).

Corpuscles 51% by volume	carry $\frac{2}{5}$ of total CO_2	in solution	0·85 c.c.
		bound (*e.g.* $NaHCO_3$)	8·45 c.c.
		adsorbed by Hb, etc.	9·7 c.c.
			19 c.c.
Plasma 49% by volume	carries $\frac{3}{5}$ of total CO_2	in solution	1·2 c.c.
		bound ($NaHCO_3$)	23·8 c.c.
		adsorbed by fibrinogen	5 c.c.
		adsorbed by serum proteins	1 c.c.
			31 c.c.

100 c.c. of Venous Blood (CO_2 tension = 45 mm., O_2 tension = 40 mm.).

Corpuscles carry	22 c.c.	= + 3 c.c. over arterial blood.
Plasma carries	34 c.c.	= + 3 c.c. ,, ,, ,,
	56 c.c.	+ 6 c.c. ,, ,, ,,

The conclusion might be drawn that some mechanism for carbon-dioxide transport in the serum, quite other than that in the cells involving haemoglobin, must be sought. The explanation of the apparent inadequacy of the latter mechanism has been found by a study of the inter-relationship between the electrolytes of serum and cell contents.

The function of serum and cell electrolytes in the transport of CO_2.

Increase in the carbon-dioxide tension of plasma causes a redistribution of chlorides as between the blood cells and the plasma, resulting in an increase in cell chloride and a decrease in plasma chloride. Some carbon-dioxide also passes into the red cells. This brings about a considerable increase in the electrolyte concentration in the cells, and, therefore, of the osmotic pressure, and water is drawn from the plasma into the cells to restore osmotic equilibrium. If the consequent increase in erythrocyte volume (*q.v.*) is taken into account, chemical analysis shows that a quantity of hydrochloric acid equivalent to the bicarbonate taken up by the plasma has passed from the plasma into the cells, and the reactions supposed to occur are as follows :—

$$NaCl + H_2CO_3 = NaHCO_3 + HCl.$$

This is followed by the passage of the liberated HCl through the cell membrane. The hydrochloric acid then reacts with the salts of haemoglobin in the same way as was previously described for carbonic acid, viz.

$$HCl + B.Hb = BCl + H.Hb.$$

This means that a considerable proportion of the respiratory carbon-dioxide (quite 90 per cent. of the total respiratory carbon-dioxide carried by the plasma) is transported in combination with bases of the plasma, which previous to the loading up with carbon dioxide were combined with hydrochloric acid. While the respiratory carbon-dioxide is " on board " this hydrochloric acid is carried in the cells in combination with base liberated from haemoglobin. *In determining the actual contribution of the haemoglobin to the transport of respiratory carbon-dioxide, it is therefore necessary to take into account not only the excess of carbon-dioxide in the cells of venous blood over that in arterial blood, but also the excess of cell chlorides.* When this is done it is found that, as stated above, the haemoglobin provides for the transport of at least 80 per cent. of the respiratory carbon-dioxide.

When the carbon-dioxide is excreted in the lung, the processes described above occur, of course, in the reverse order, although, owing to the fact that under all physiological conditions plasma contains a higher concentration of both chlorides and carbonates than the cells, this involves the passage of chlorides and carbonates from a lower to a higher concentration.

This simple explanation of the mechanism of the transport of carbon-dioxide needs, therefore, further exploration, since it would seem to entail first the displacement of the strong acid hydrochloric by the weak acid carbonic in the plasma, and then later the replacement in the cells of the relatively stronger acids hydrochloric and carbonic by the very weak acid haemoglobin (which even in its oxygenated form is still a very weak acid). *It is well known that a weak acid will replace a strong one in combination if the strong one is removed as it is formed,* and, as indicated above, it is on this basis that the removal of carbon-dioxide from the blood, in the lung, is explained. But it is necessary to consider why the displacement of chlorides should take place, since there is no variation in chloride tension in lungs and tissues.

It has been stated that carbonic acid displaces the stronger hydrochloric acid from combination as the chlorides of the plasma, because the hydrochloric acid so liberated diffuses through the cell membrane and is thus removed from the sphere of action, and that therefore the reaction $NaCl + H_2CO_3 \rightleftharpoons NaHCO_3 + HCl$ proceeds from left to right. It must, however, be remembered that the cell membrane is freely permeable also to carbon-dioxide, and that, in fact, at least 40 per cent. of respiratory carbon-dioxide passes into the cell and there displaces base from combination with haemoglobin. Since, moreover, enough base can be liberated from combination with haemoglobin to combine with the whole of the

respiratory carbon-dioxide, it is obvious that some other factor must be involved which determines how much carbon-dioxide shall enter the red corpuscle and how much shall react with chloride in the plasma, liberating hydrochloric acid, which then enters the red cell.

Since the passage of carbon-dioxide into the cell under increased carbon-dioxide tensions, and the passage of carbon-dioxide out of the cell under lowered carbon-dioxide tensions, is accompanied by a transfer of chlorides in the same direction, it would seem that the distribution of one of these electrolytes is related to the distribution of the other. For information on this point, the distribution of all the main solutes of the blood between plasma and cells must be studied. It is then found that :—

(1) The solutes of the blood are so distributed that the red cell contents and plasma are isotonic (*q.v.*). This is indicated by the shape of the cells (Chap. XXII.).

(2) The red cells are impermeable under physiological conditions to the bases of the blood (excepting the hydrion).

(3) The red cells are freely permeable to the non-colloidal anions of the blood.

(4) The red cells are impermeable to the protein anions of the cells and plasma.

From Donnan's theory of membrane equilibria (*q.v.*) it may be deduced that under these four conditions, for thermodynamic equilibrium

$$\frac{[Cl_c]}{[Cl_s]} = \frac{[HCO_{3c}]}{[HCO_{3s}]} = \frac{[H_s]}{[H_c]} = \frac{[A_c]}{[A_s]}$$

Where $[A_c]$ is the ionic concentration of anions in the cell.

$[A_s]$ is the ionic concentration of anions in the serum, etc.

The ratio $\frac{[A_c]}{[A_s]}$ has been called r.

Thus it is seen that when carbon-dioxide enters the blood, thus altering the ratio $\frac{[HCO_{3c}]}{[HCO_{3s}]}$, a re-distribution of the other monovalent anions (mainly chlorides) must occur *to preserve thermodynamic equilibrium.* But this is not the only condition governing the electrolyte distribution in blood. *For osmotic equilibrium* the total osmolar concentration in plasma and cells must be equal, and therefore the passage of carbon-dioxide and chloride from plasma to cells must be accompanied by a passage of water in the same direction, with a consequent increase in cell volume. *Also for electrical equilibrium* the total positive ions of the serum must equal

the total negative ions of the serum, and likewise for the cells. These three conditions absolutely determine the relative distribution of electrolytes under given conditions. If the comparatively low osmotic pressures of haemoglobin and serum protein are ignored, an approximate value for r may be deduced, viz.:

$$r = 1 - \frac{[\mathrm{Hb}_c]}{2\,[\mathrm{A}_s]}.$$

As $\frac{[\mathrm{Hb}_c]}{2\,[\mathrm{A}_s]}$ cannot be negative, r must always be less than 1, *i.e.* there will always be an unequal distribution of anions about the membrane, the concentration in the cells being always less than that in the serum.

Hence the transport of respiratory carbon-dioxide in the blood involves the passage of carbonate, chloride and water from plasma to cells, a change in cell volume " v," a change in the anion ratio " r " and a slight change in hydrogen ion concentration; the changes which occur when the haemoglobin is reduced and the carbon-dioxide is picked up occur in the reverse order when the haemoglobin is oxygenated and the carbon-dioxide excreted. Thus it may be seen that in determining equilibrium conditions in the blood, it is necessary to consider oxygen tension, carbon-dioxide tension, pH, r, v, the percentage of haemoglobin combined with oxygen, and the percentage of carbon-dioxide free and in combination with base as bicarbonate. The relationship between any two of these factors, when other conditions are constant, may be represented by curves.

In studying the mechanism of the gaseous interchange of blood, it is convenient to begin by studying the reaction of blood *in vitro*, under specified conditions. From such studies, curves like Fig. 82 may be deduced. These curves do not, however, at all represent the gaseous exchange of blood *in the body*. They represent the variation of one gaseous constituent (*e.g.* O_2) with one other condition (*e.g.* oxygen pressure), assuming that other conditions (*e.g.* tensions of other gases) are constant. In the circulating blood, however, in lungs and tissues, not only oxygen tension, but carbon-dioxide tension, vary from point to point, and the oxygen absorption curve for blood *in vivo* is one which *intersects* the curves representing oxygen absorption at different oxygen pressures under different constant carbon-dioxide tensions of physiological range.

To define the complete equilibrium conditions of blood at any point a large number of curves would be necessary, indicating the relationship between oxygen and carbon-dioxide content and ten-

sion, hydrogen ion concentration, free and bound carbon-dioxide, *r*, cell volume, etc. It has, however, been shown that for any given sample of blood an "*alignment chart*" may be drawn, and graduated in such a way that each curve on it represents one variable factor in the blood, and a straight line drawn to cut all the curves will do so at points which give the value of each of the respective factors in the blood at any one time. Hence, if the simultaneous values of any two factors in the blood be known, all the others may be deduced from the chart by joining the points

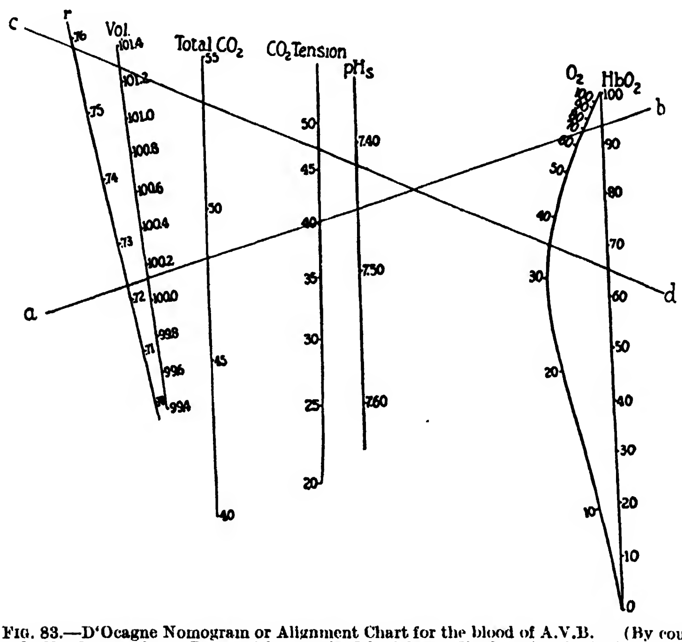


FIG. 83.—D'Ocagne Nomogram or Alignment Chart for the blood of A.V.B. (By courtesy of L. J. Henderson, from *Recent Advances in Physiology*. Explanation in text and also in *Lectures on Certain Aspects of Biochemistry*.)

corresponding to these values on their respective curves and producing the line to intersect the other curves.

In the alignment chart (Fig. 83) the seven graduated lines, taken from left to right, represent respectively (1) the ratio, $r =$ (anions of cell)/(anions of serum); (2) the volume of the erythrocytes, v; (3) the *total* CO_2 of the blood; (4) the CO_2 *tension*; (5) the pH of the serum; (6) the oxygen tension; and (7) the percentage saturation of haemoglobin with oxygen. If any two of these values are known for the blood for which an alignment chart has been constructed, then the five other concomitant values may be obtained by drawing a line through the two known points. This line will cut all seven lines, and the point at which it cuts a

graduated line will give the value for that factor. For example, in Fig. 88, which is the alignment chart for A.V.B.'s blood, if one knew that, at any time, the blood, under a tension of 40 mm. Hg of CO_2, would give a serum with a pH of 7·45, then, by joining these points and producing the line to cut the other curves (*i.e.* the line *a*—*b* in the figure) the values of r, v, total CO_2, O_2 tension, and percentage saturation of Hb with oxygen are given by the points of intersection of *a*—*b* with the respective graduated curves. This shows us that when this blood has a CO_2 tension of 40 mm. Hg, and its serum a pH of 7·45, the chlorides and bicarbonates are so divided between cells and serum that $[BHCO_3]_c/[BHCO_3]_s = [Cl]_c/[Cl]_s = r = 0{\cdot}723$; the cell volume, v = vol = 100·1; the total CO_2 = 48 per cent.; the oxygen tension = 70 mm. Hg; and the percentage saturation of haemoglobin with oxygen = 92.

A word of explanation is necessary concerning the value (vol) of the cell-volume. Professor L. J. Henderson, to whom we are indebted for the chart, took the normal cell-volume ($70\mu^3$) as 100 per cent., when the O_2 tension was 80 mm. Hg and the CO_2 tension was 39 mm. Hg. At this point the haematocrite reading was 40 per cent., *i.e.* the cells occupied two-fifths of the total blood volume and the plasma the remaining three-fifths.

The line *a*—*b* represents a typical set of conditions for arterial blood. Another line, *c*—*d*, could be drawn to represent the values of the seven factors in typical venous blood. Here the CO_2 tension has risen to 47·5 mm. Hg, and the Hb saturation has fallen to 65 per cent. It is evident that this produces marked alterations in the values of r and v, and a less marked increase in hydrogen ion concentration. Between the extremes represented by the oxygen and carbon-dioxide tensions indicated by the lines *a*—*b* and *c*—*d*, *i.e.* between arterial and venous conditions, a large number of lines are possible, representing the conditions in capillary blood. For example, blood with an oxygen tension of about 60 mm. Hg. might pass through a very active tissue producing large quantities of CO_2. The blood as it reached this tissue might have the following characteristics, viz. $r = 0{\cdot}73$; vol. = 100·4; total CO_2 = 48 per cent.; CO_2 tension = 42 mm. Hg; pH (serum) = 7·41; and percentage HbO_2 = 90 per cent. Interchanges with the active tissue would cause a decrease in factors 6 and 7 on the chart, and an increase in the factors represented by the first five lines, *e.g.* $r = 0{\cdot}75$; vol. = 101; total CO_2 = 52·5 per cent.; CO_2 tension = 45; pH serum = 7·42, all increase; while O_2 tension = 36 mm. Hg and HbO_2 per cent. = 70, show a decrease. Oxygen has been given to the tissues, and CO_2 accepted from the tissues with a

consequent adjustment of r, vol and pH_s to meet the new conditions. Thus, by this simple diagram, the complicated changes occurring in the blood during respiration or following on the activity of any organ, can be depicted.

It must be noted that each alignment chart represents the conditions in the sample of blood from the analysis of which it was constructed, and must not be applied generally to any blood under any conditions. Any change of condition necessitates the construction of a new chart.

Integrative action of blood plasma.

Plasma must be considered as a solution of water in colloid separated from other fluids (with which it is in equilibrium) by membranes permeable to certain solutes. The whole transport system is a multi-phase colloidal complex in equilibrium. Alterations in any phase produce regulatory changes in every other phase. Briefly, *blood has an integrative action.*

To come back to the simile of a community: suppose Cottonopolis failed to function normally. This would be manifested by the scarcity of cotton goods in the hands of the distributors. The cause of the failure might be found by a process of elimination. In general, (1) either the supply of raw material was inadequate (bad harvest or transport strike), (2) or the supply of fuel was restricted (coal strike), (3) or the workers were on strike, or (4) the means of distributing the finished product had broken down (transport strike). It might even happen that (5) over-production had " drugged " the market, producing the invariable reaction on the factory. Similar mishaps might overtake that collection of cell-communities called the animal organism. (1) If the various raw materials are not available even when ample fuel supplies exist, cell life becomes narrowed and inefficient. Certain matter must be imported—it cannot be manufactured. If the raw material is imported but does not reach the cell, then the transport system is at fault. (2) A similar statement could be made about the supply of energy. (3) The cell itself may be at fault—*e.g.* after HCN poisoning, in spite of adequate supplies of energy and material, metabolism is at a low ebb. (4) The transport trouble might be due to the scarcity of barges, *e.g.* anaemia, or to want of force in the driving mechanism, *e.g.* heart failure. (5) In certain pathological conditions a cell-community may take the bit between its teeth and overproduce. The immediate result is to hamper its own activities by the presence of the products of its activity.

To take a specific instance—suppose we find that an organ seems to suffer from a lack of oxygen. This may be due (i.) to

a scarcity of oxygen in the air breathed—analysis will show that. (ii.) The lung mechanism may be out of order (Chap. XXVII.). (iii.) The membrane separating lung-air from blood may have lost its permeability. Comparison of the oxygen capacity of arterial blood with its actual oxygen content will indicate whether or not this is the fault. (iv.) This will also show if the erythrocytes are taking on their full load. (v.) If the blood suffers little or no desaturation on passing through the organ, then one may presume either that the haemoglobin has lost its power of unloading oxygen (methaemoglobin) or that the organ has lost the power of using oxygen. Examination of the blood pigment by means of the spectroscope may help us to choose which of these alternatives is correct.

No matter what organ or tissue it is that fails to function normally—it must remain in dynamic equilibrium with the blood, and through the blood with every other tissue and organ in the body. Every change occurring anywhere in the body sets in motion a series of far-reaching alterations tending to restore equilibrium. It is this constant adjustment of physical and physico-chemical force brought about mainly *viâ* the inland transport system that goes to make up the metabolism of the organism.

Further Reading

Barcroft. "The Respiratory Function of the Blood." Cambridge University Press.

L. J. Henderson. In "Certain Aspects of Biochemistry." University of London Press.

Van Slyke. "Factors Affecting the Distribution of Electrolytes, Water and Gases in the Animal Body." Lippincott.

CHAPTER XXIV

LOADING UP

> " I send it through the rivers of your blood
> Even to the court, the heart, the seat o' the brain,
> And through the cranks and offices of man,
> The strongest nerves and small inferior veins
> From me receive that natural competency
> Whereby they live." SHAKESPEARE.

WE have now come to one of the most interesting parts of our study, namely the handling of the imports in their course between the external and the internal transport systems. As we have seen, the material brought to the body may be divided into two classes. One of these consists of the gas, oxygen, which comes to the port of arrival almost ready for use, and which is passed directly to the inland transport system for transmission to the various cell-communities.

The foodstuffs form the other class. They are " raw material " and have, as a rule, to undergo some process of manufacture before they can be distributed to the consumer. They are handled by a special mechanical transport service and are taken through the various factories and then handed to the inland transport.

In this chapter we are to deal with the importation of oxygen and the mechanism by which it is received at the port, carried overland and loaded on the submersed barges on their way inland. Indissolubly associated with any system of importation is the provision of exports. Any barge travelling empty on the blood stream as on any industrial canal is a distinct loss to the whole community. Every ship that leaves our shores without a full cargo tells a tale of industrial inefficiency. In the body, the output of carbon-dioxide and the intake of oxygen are nicely balanced. As a matter of fact, the regulation of the rate of importation by the rate of exportation is as much a law here as in the realm of Political Economy.

A separate chapter has been devoted to the mechanism whereby the oxygen is brought from outside into the port. Briefly, by muscular movements air is drawn through filtering and warming appliances into the air sacs, and after a very short interval is expelled into the outer air altered in content.

In the average resting man, somewhat over 500 c.c. of air come

into the respiratory chambers at each ordinary quiet inspiration, and somewhat less than this amount is expelled at each expiration. One may say, in round numbers, that the **tidal air** of the average adult is about 500 c.c. The tidal air at various ages and weights is given below (Table L.):

TABLE L

Age.	Weight in Kilos.	Tidal air in c.c.
0–6 months.	3·77	48
6–12 months.	7·7–12	85–130
3– 7 years.	14·3–19	124–220
8–14 years.	22–29	220–400
Adult.	60–70	300–550

If a very deep inspiration is taken, more than 500 c.c. can be sucked into the lungs. This extra quantity, which varies with the " build " and expertness of the subject, but which usually is about three times the tidal volume, is called the **complemental air**. By a forced expiration after a *normal* expiration the **reserve air** may be expelled from the lungs. The volume so expelled varies very much, and may markedly be increased by practice. Some people can breathe out only an additional 500 to 700 c.c., while singers, physical training experts and others who practise abdomino-thoracic breathing, may register a volume of 1,500 to 2,500 c.c. These three quantities together give the **vital capacity** of an individual, *i.e.* the amount of air that a person can expire after a *deep* inspiration. It is not possible completely to empty the lungs. As we shall see later, a mechanism exists in the air vesicles which prevents their total collapse. They retain about a litre of air—the **residual air**.

To summarise, taking average figures :

Vital capacity	Tidal air	500 c.c.
	Complemental air	1,500 c.c.
	Reserve air	1,500 c.c.
		3,500 c.c.
Residual air		1,000 c.c.
Volume of fully distended respiratory apparatus		4,500 c.c.

This volume of 4,500 c.c. includes not only the volume of the lungs but of the approaches to the lungs—the nasal cavity, the trachea, the bronchi and the bronchioles. These constitute the

outer harbour or "**dead space,**" which has a capacity of about 140 c.c. That is, in ordinary quiet breathing each respiration brings about 360 c.c. of air into the inner harbours (air sacs, alveolar sacs, or **infundibula**). These funnel-like chambers to which the air passages lead are the most expansile structures in the lung, and they are largest where the expansion of the lung is greatest. All round their walls open myriads of small thin-walled air-cells or **alveoli**—the true wharves of the port. There and there alone takes place the interchange of exported CO_2 and imported O_2.

Let us look first at the **area of wharfage**. The interior of the air sacs and their alveoli is lined by a thin transparent layer of endothelium. If the lining could be stripped from all the sacs of both lungs and inflated, it would form a spherical balloon about 17 feet in diameter. If it were spread as a continuous flat sheet it would cover a square floor of 30 feet by 30 feet. In other words, the area of wharfage is, at least, over fifty times the surface area of the body. The average diameter of an air sac is 0·2 mm., with a volume of 0·004 cub. mm., and an area of 0·125 sq. mm. Suppose these air cells to be spherical and closely packed together, then the maximum number contained in a cubic millimetre of lung substance would be 250 cells of total surface 31·2 sq. mm. Now the average value for the total volume of lung substance is 1617 c.c. This provides for the possible presence of 404,500,000 air sacs, with a surface of 50·56 square metres. Of course this is a *maximum* value for the *number*. From the volume of lung substance has to be deducted the volume of the supporting cells of the lung, of the blood vessels, and of the air passages. On the other hand, a *minimal* value is given for *area*, since no account is taken of the increase of surface caused by the projection of the blood capillaries into the lumina of the alveoli. Various estimates have been made of the surface area of the alveoli, ranging from that of von Huschke of 2,000 sq. metres to that of Äeby given above. Hufner's value is generally taken as a mean, viz. 140 sq. metres. Of this area, about three-fourths consists of thin-walled capillary blood vessels. That is, the **effective absorptive surface** is about 100 sq. metres.

Over a surface of about 100 sq. metres, interchange between alveolar air and blood is possible. Just behind this surface-epithelium lie capillary blood vessels of such small bore that the red blood corpuscles are distorted in their passage through them. This naturally produces a decrease in the rate of blood flow. The rate is further decreased by the increase in the total sectional area of this capillary system, which is at least seven times greater than that of the aorta (Chap. XXV.). The sudden increase in the

area over which the blood has to spread itself in a layer less than one corpuscle thick causes a marked decrease in the velocity of the stream. These two conditions, (*a*) narrow bore, and (*b*) increased area of distribution, of course facilitate the processes of unloading and reloading the erythrocytes. The structure is, in principle, just the same as that of the kidney.

The next problem before us is that of the **transference of carbon-dioxide** from the blood to the air and of the **oxygen** from the alveolar air to the blood. About the first process there seems to be no difficulty. Everyone is agreed that, as the tension of carbon-dioxide in the blood of the pulmonary artery just as it enters the capillary system is greater than its tension in expired air, a simple process of diffusion through a wet membrane is all that is required. The tension of CO_2 in alveolar air and in the blood is 40 and 46 mm. Hg respectively. There is, therefore, a difference of 6 mm. Hg in the CO_2 pressure tending to cause a flow of CO_2 from blood to air. Is this gradient of pressure sufficient to account for the 250 c.c. of gas normally expired per minute ?

The passage of gas through a membrane depends (*a*) on the nature of the membrane, (*b*) on the structure of the membrane, (*c*) on the physical state of the membrane, (*d*) on the nature of the gas, and (*e*) on the gradient of pressure.

(*a*) The two layers of flattened cells separating blood from alveolar air differ little in chemical nature from any other similar structure. *One may note, however, their richness in lipoids.* (*b*) They are constructed of large irregular flattened cells forming an extremely delicate layer as thin as the film of a soap bubble. The average thickness of the membranous layer is 0·004 mm. (*c*) Not only does the protoplasm forming the membrane contain about 90 per cent. of water dispersed through it, but its surface is kept moist on both sides. (*d*) Carbon-dioxide is very soluble in water, and more soluble in lipoid. Water at body temperature and atmospheric pressure will absorb over half its volume of carbon-dioxide. (*e*) Experiments by Krogh and others seem to have proved beyond question that the differences in tension existing on the two sides of the lung tissue are quite sufficient to account for the passage of the necessary volume of gas.

It is worth while to look a little more closely at this problem. In Chap. XXIII. is given a table (XLV.) of absorption coefficients of the respiratory gases. These values of α indicate the volumes of gas at N.T.P. which will dissolve in 1 c.c. of water. Later in the same chapter, figures which hardly differ from α were given for the solubility of these gases in plasma, etc. The velocity of diffusion depends not merely on the pressure gradient and on the

absorption coefficient of the gas, but also on a factor **k**—the **diffusion coefficient. k** is a constant for each gas and each temperature. The appended Table LI. (from Loewy) will amplify this.

TABLE LI

DIFFUSION COEFFICIENTS

Temperature	16° C.	37° C.
Oxygen	1·62	1·68
Carbon-dioxide	1·38	1·43
Nitrogen	1·73	1·79

The product of α and **k** gives the diffusion rate in cm. per 24 hours through a layer of water, 1 cm. × 1 sq. cm. with a pressure gradient of 1 atmos. For example, at 37° C. carbon-dioxide has a diffusion rate of 1·43 × 0·57 = 0·815 cm. per 24 hours.

It has been found that **k** bears a definite inverse relationship to the square root of the molecular weight of the gas. The result of multiplying the diffusion coefficient by the square root of the molecular weight of the gas is thus a constant for all gases. This diffusion factor $k\sqrt{m}$ has a value, for water, of 0·0649.

The diffusion rate through lung substance, because of its large content of lipoids and lipins, must be greater than that through water. Experiments with soap bubbles and with frogs' lungs have confirmed this deduction. It has also been found that the velocity of diffusion is absolutely unaltered by slight alterations in the pH of the lung tissue. Loewy maintains that the rate of diffusion is the same in dead and in living lung tissue. The diffusion factor through lung has been estimated as 0·139. Experiment has shown definitely that CO_2 passes just as readily in either direction through the lung wall. This has been amply confirmed by Krogh, who found that the direction of diffusion depended entirely on the direction of the gradient of pressure, and the rate of diffusion was regulated by the steepness of this gradient.

The volume of gas diffusing per minute through 1 sq. cm. of alveolar wall may be calculated from this formula :

$$v = \frac{\alpha(p_1 - p_2)\,C}{760\,\sqrt{m}\cdot d}.$$

Dealing with carbon-dioxide we may evaluate as follows :

α at 37° = 0·57,

p_1 = CO_2 tension in the blood of the pulmonary artery = about 46 mm. Hg.

p_2 = CO_2 tension in alveolar air = about 40 mm. Hg,

$p_1 - p_2$ = 46 − 40 = 6 mm. Hg.

This difference of pressure, of course, only exists at the beginning of the experiment. The blood loses carbon-dioxide, *i.e.* p_1 *decreases*; CO_2 passes into the alveolar air, *i.e.* p_2 *increases*, and $p_1 - p_2$ tends towards zero. It is, therefore, necessary to take a mean value between 6 and 0, *i.e.* 3 *mm. Hg.*

$$C = \text{diffusion factor} = 0{\cdot}139,$$
$$\sqrt{m} = \sqrt{44} = 6{\cdot}63,$$
$$d = \text{thickness of alveolar wall} = 0{\cdot}004 \text{ mm.},$$

i.e.
$$v = \frac{0{\cdot}57 \times 3 \times 0{\cdot}139}{760 \times 6{\cdot}63 \times 0{\cdot}004}$$
$$= 0{\cdot}01 \text{ c.c. per minute.}$$

As the effective absorptive surface of the lung is about 100 sq. metres, there can pass through it each minute

$$100 \times 10{,}000 \times 0{\cdot}01 = 10{,}000 \text{ c.c.}$$

of carbon-dioxide by simple diffusion.

One may consider the problem from another aspect and determine the gradient of pressure necessary to furnish the 250 c.c. of carbon-dioxide normally expired per minute. Transposing the formula, one gets

$$p_1 - p_2 = \frac{v \times 760 \sqrt{m} \times d}{\alpha \times c}.$$

Evaluating this,

$$p_1 - p_2 = \frac{250 \times 760 \times 6{\cdot}63 \times 0{\cdot}004}{0{\cdot}57 \times 0{\cdot}139 \times 100 \times 10{,}000}$$
$$= 0{\cdot}063 \text{ mm. Hg.}$$

That is, a difference of CO_2 tension between blood and alveolar air of only $2 \times 0{\cdot}063 = 0{\cdot}12$ mm. Hg would be quite sufficient to cause 250 c.c. of CO_2 to pass through the lung wall per minute. During work the amount of carbon-dioxide eliminated by the lungs may be increased *tenfold.* The above figures show that there is ample wharf-space for this exportation.

The transference of oxygen from alveolar air to blood has been the cause of much controversy. Two conflicting views both backed by experimental facts are held.

(1) The lungs may be considered as secretory glands. Fish have a swim-bladder which is, like the lungs, an outgrowth from the alimentary canal. Oxygen is secreted by it so as to equalise the specific gravities of fish and water. The fish may secrete oxygen against the pressure produced in the bladder by immersion to a great depth, *e.g.* against the pressure of hundreds of atmospheres. Against this view may be opposed the histological fact that cells composing the walls of the swim-bladder structurally do

not resemble those of the lung. The former are deep granular cells typical of secretory tissue, while the latter, like the capsule of Bowman in the kidney, are thin and flat. Moreover, birds, which have, of all animals, the most rapid and efficient respiratory exchange and so should have a lung epithelium exhibiting marked secretory qualities, have no epithelial covering at all, so that the capillaries appear to be almost completely free and surrounded by alveolar air.

(2) Most modern workers maintain that just as CO_2 diffuses outwards, so does oxygen diffuse from air to blood. The whole controversy turns on the existence of a pressure gradient for oxygen. The earlier investigators got results which indicated that the oxygen tension of the blood frequently exceeded that of the alveolar air. Later workers like Douglas and Haldane disagree with the earlier findings, and by the employment of finer technique have proved definitely that normally the tension of oxygen is always less in the blood than in the alveolar air. They still maintain, however, that under certain more or less abnormal conditions—say, acclimatisation to high altitudes—there is an active absorption and transference of oxygen to the blood on the part of the pulmonary epithelium.

A man at rest requires about 300 c.c. of oxygen per kilo of body weight per hour. The average man weighs about 66 kilos, *i.e.* 330 c.c. of oxygen must pass into his blood every minute. During violent exercise the necessary intake of oxygen may be as great as 3,000 c.c. per minute. In order to produce this transference from air to blood a certain pressure difference is necessary.

Krogh has shown by an ingenious tonometric method that the oxygen tension of the blood is always lower than the alveolar oxygen tension, and the difference is generally 1 to 2—even 3 to 4—per cent. of an atmosphere. One must now consider whether 1 per cent., *i.e.* 7·6 mm. Hg, is a sufficient pressure gradient for respiratory purposes.

Employing the same formula as for CO_2, one finds with a difference of pressure of 7·6 mm. Hg that

$$v = \frac{0{\cdot}0239 \times 3{\cdot}8 \times 0{\cdot}139}{760 \times 5{\cdot}66 \times 0{\cdot}004} = 0{\cdot}0006 \text{ c.c.}$$

per minute per sq. cm. This gives a value of

$$100 \times 10{,}000 \times 0{\cdot}0006 = 600 \text{ c.c.}$$

passing through the total effective absorptive surface of the lung. Thus we see that the physical conditions allow for an ample supply

of oxygen for ordinary purposes. As a matter of fact a difference in pressure of less than 4 mm. would be quite sufficient to ensure the supply of the 330 c.c. per minute required by the average man resting but awake.

How is the rate of transference increased to meet the needs of the man doing hard muscular work who uses up 3,000 c.c. of oxygen per minute? One very obvious point of difference between a resting and a working man lies in the volume of air passing into the lungs per unit of time. The following table (LII.) shows that the ventilation of the lungs is markedly increased by the performance of work.

TABLE LII

VENTILATION OF LUNGS

	Litres per min.	Respir. per min.
Resting	6–7	13–14
Walking	24	14
Running	60	15
Swimming in cold water	90	—
Running up and down stairs (greatest possible effort of a noted swimmer)	190	over 60

That is, by the constant addition of fresh air to the lungs the tension of oxygen in the alveoli is kept from falling. A tenfold increase in ventilation provides an ample margin for even the most strenuous work.

Those who hold to the secretory hypothesis maintain that while diffusion is capable of providing a sufficient oxygen supply for a normal existence even with hard muscular work, yet when the pressure of oxygen in the lung is brought much below normal, active secretion by the lung epithelium must be brought into play. Aviators, for instance, rise to great heights, and so come under a low barometric pressure.

TABLE LIII

EFFECT OF HEIGHT ON BAROMETRIC PRESSURE

Height above sea level in metres.	Barometer mm. Hg.	Per cent. of an atmosphere.
0	760	100
1,000	670	88
2,000	593	78
3,000	524	69
4,000	463	61
5,000	410	54
6,000	357	47
7,000	320	42

It is well known that ballooning, for instance, causes respiratory distress; so, too, does mountaineering. Mountain sickness fre-

quently begins at altitudes of 2,000 to 3,000 metres, particularly if the ascent has been fairly rapid by railway. Airmen usually suffer after ascending 5,000 to 6,000 metres. The partial pressure of oxygen in the blood as determined by the carbon-monoxide method has been found to be 35 mm. Hg above the oxygen pressure of alveolar air. Considerable doubt exists, however, as to the validity of this method. It depends on the careful matching of a carboxylated blood with a blood-carmine mixture, and minute quantities of blood are used.

TABLE LIV

EFFECT OF ATMOSPHERIC PRESSURE ON ALVEOLAR OXYGEN TENSION

	Height above sea level in metres.	O_2 tension of air. mm. Hg.	Alveolar O_2 tension. mm. Hg.
Berlin	54	157	105
Brienz	500	148	88
Brienzer Rothorn	2,130	121	62
Col d'Olen	2,900	110	60
Monte Rosa	4,560	89	61

One fact in the data given by those investigators is a little strange although it may have no significance. Notwithstanding that the arterial oxygen tension was always higher than that given for the alveolar air, it was never higher than that of the atmosphere at the time, although occasionally not much below it. Why should the secretory power fail just at this level and not raise the oxygen tension above that of the atmosphere? Is it possible that the blood had come into equilibrium with an oxygen tension which was not given correctly by the measurement of that of the alveolar air? Might it not also be possible that the carbon-monoxide method gives different values when the haemoglobin content of the blood is increased, as in the case of acclimatisation to high altitudes?

TABLE LV

ATMOSPHERIC PRESSURE AND NUMBER OF ERYTHROCYTES

	Height above sea level in metres.	Red corpuscles.
Christiania	0	4,970,000
Zurich	412	5,752,000
Davos	1,560	6,551,000
Arosa	1,800	7,000,000
Cordilleras	4,392	8,000,000

Hasselbalch shows that the hydrogen ion concentration is increased under those circumstances.

"This question of secretion by the lungs is instructive from the point of view of 'vitalism.' When first proposed, it was held to apply to the

ordinary state of affairs; but as improvements were made in experimental methods, the absorption was shown to follow physical lines: it was then held to apply to cases of muscular exercise, and now only to acclimatisation to high altitudes. One might venture to say that the more accurate the methods of investigation the better is it found that chemical and physical laws are capable of explaining physiological phenomena."—Bayliss, *Principles of General Physiology.*

Let us now consider what happens to the inland transport service when the port becomes congested with incoming traffic. Compressed air is used in all the great sub-aqueous works of to-day, in diving, in preparing foundations for bridges, in pier building, and in the construction of tunnels or shafts through water-bearing strata. It is well known that a large percentage of the men working under those conditions suffer illness and many die. In the construction of the Adour bridge 90 per cent. of the workers suffered from " compressed air " disease, and in the boring of the Hudson Tunnel 2 per cent. of the caisson workers died each month. Compressed air sickness is characterised by its protean symptoms —loss of speech, blindness, deafness, transitory madness, vertigo, loss of conciousness, emphysema, spinal paralysis, etc. None of the symptoms, with the exception of some slight ear trouble, ever occurs while the men are under pressure. " Mules lived about a year in the Hudson tunnel and were healthy enough to kick and bite at all comers." The illness seemed to come on during or after decompression, and is now known to be due to the appearance of bubbles of nitrogen in the tissues. Boyle, in the seventeenth century, showed that bubbles of gas appeared in the humours of a viper's eye when submitted to rapid decrease of air pressure under an air pump. Paul Bert in a remarkable series of experiments (1870–1880) proved that these bubbles were nitrogen and that they might block up the capillaries in some part of the body and, by cutting off that part from the blood supply, produce one or other of the symptoms mentioned above.

If merely the *pressure* of the surrounding air is increased, why should nitrogen alone be set free on decompression? When a person is placed in compressed air, the blood passing through the lungs dissolves the same *volume* of the atmospheric gases as it does under normal conditions, but the *weight* of gas absorbed will be increased above normal in proportion to the increase in partial pressure of each gas in the alveolar air. Now we have seen that the partial pressure of carbon-dioxide in alveolar air is a constant, hence there can be no increase in the amount of carbon-dioxide present in the blood during exposure to compressed air. Oxygen is carried in two ways, (*a*) by haemoglobin, and (*b*) in

simple solution in the plasma. (*a*) At atmospheric pressure the haemoglobin is almost saturated with oxygen—the little erythrocyte barges are comfortably filled. Increase of alveolar tension *may* produce a slightly better oxygenation of the haemoglobin, but it requires a very marked increase of pressure to make an appreciable increase in the amount of oxygen carried by this means (Fig. 82). (*b*) According to Dalton's Law, the amount of gas dissolved is directly proportional to its partial pressure.

At body temperature and normal pressure, arterial blood holds 3 c.c. of oxygen in solution in every litre of fluid. If the pressure is increased x times, then each litre will still dissolve 3 c.c. of oxygen, but this oxygen will weigh x times as much as normally. On being carried to the tissues, the blood will share its dissolved oxygen with them in proportion to its partial pressure and to its solubility in the various tissues. These tissues will use up the dissolved oxygen in preference to that carried by the corpuscles, and as the amount in solution, except after exposure to enormous pressures, is only a small percentage of the total available oxygen in the arterial blood, it will soon be used up. *We again draw attention to the fact that increase in the available oxygen does not cause increase in its utilisation by the cell.* A candle burns more brightly in oxygen and soon ends its light-giving career. The cell " ca's canny "—holds on the even tenor of its way, takes up the oxygen it requires for its immediate needs and keeps no store but the tiny quantity dissolved in its protoplasm.

To take a concrete example. At 38° C. and atmospheric pressure 1 litre of blood contains 200 c.c. of oxygen carried by haemoglobin and only 3 c.c. in simple solution (measured at N.T.P.). Sixfold increase of pressure makes no appreciable difference to the value of the corpuscular oxygen, but increases the dissolved oxygen to about 18 c.c. That is, the ratio of dissolved to " bound " oxygen is increased from about 1/70 to 6/70. The entire result would be that as there are about 3½ litres of blood in the average man the *venous blood* would carry not more than 20 per cent. more oxygen than normally. In other words, the desaturation of haemoglobin would take place to quite the same extent as under atmospheric pressure. This eliminates oxygen as the gas causing " compression illness " and leaves nitrogen alone to be dealt with.

Let us first consider how the nitrogen taken up by the blood from the alveolar air is distributed to the various tissues of the body. In view of what we have seen as to the ease and completeness with which the blood becomes saturated with oxygen in its passage through the pulmonary capillaries, we may take for granted that saturation with nitrogen under the same conditions

is just as complete. It is also reasonable to suppose that Dalton's Law of partial pressures is just as applicable to blood as to any ordinary solution exposed to compressed air. This supposition is supported by experimental facts. When blood is exposed to compressed air it will absorb a volume of nitrogen commensurate with the absorption coefficient of this gas in blood. During its passage through the tissues it will share its load of nitrogen with them in proportion (*a*) to the absorption coefficient of the gas in blood and tissues and (*b*) to the partial pressure of the gas in blood and tissues. (*a*) With regard to the first point, the solubility of nitrogen per unit mass of tissue varies greatly. For example, fat can absorb about six times as much nitrogen gas as blood, while the earthy constituents of bone probably absorb only an infinitesimal amount. With these two tissues excepted we may consider that, as the others differ but slightly in chemical and physical constitution from plasma, they also take up approximately the same quantities of gas. (*b*) Normally the tissues are saturated with nitrogen at its partial pressure in the atmosphere—every gram of tissue contains approximately 0·0145 c.c. of nitrogen. If the external pressure is increased, this volume will immediately be diminished correspondingly, and the deficit will be made good at the expense of the circulating blood. Take for example the sudden increase in pressure to 3 atmospheres brought about by a rapid descent through 60 ft. of water to the bed of the sea. The volume of gas in solution in the body is at once reduced to one-third, viz. 0·005 c.c. per gram. At the same time the blood in the lungs has its content of nitrogen reduced from its normal value of 0·87 c.c. per 100 c.c. of blood to 0·29, with almost immediate restoration to the normal figure. The litre of blood in the capillaries of the lungs would now have in solution three times the weight of nitrogen as under normal pressure. When the blood arrives at the tissues, partition of its load will take place. Each gram of tissue has a deficit of 0·01 c.c. of nitrogen, and nitrogen will pass from blood to tissue till each gram of tissue contains the normal value of 0·015 c.c. per gram. This value will not be reached at once, because the very acquisition of nitrogen by the tissue implies the loss of nitrogen by the blood. The blood then returns to the lungs for a fresh charge, which it again shares with the tissues, and so on. Haldane calculates that somewhere about five hours are required before the body is completely saturated with nitrogen after any change of pressure, *i.e.* till the partial pressure of nitrogen in the tissues corresponds with its partial pressure in the blood and so to its partial pressure in the alveolar air.

If we consider the amounts taken up by the various tissues we may arrive at some conclusion as to the mechanics of the processes of saturation and desaturation. The average working man weighs 70 kg.; of this amount 15 per cent., or 10·5 kg., is fat or fatty material; 5 per cent., or 3·5 kg., represents the amount of blood; while the earthy constituents of bone (about 3 per cent.) may be neglected.

DISTRIBUTION OF NITROGEN IN THE TISSUES OF MEN WEIGHING 70 KG.

Tissue.	Per cent. of body wt.	Wt. of tissue.	Vol. of nitrogen.
Blood	5	3·5 kg.	30 c.c.
Fat	15	10·5 ,,	530 ,,
Bone	3	2·1 ,,	0 ,,
Residue	77	53·9 ,,	435 ,,
	100	70 kg.	995 c.c.

Blood, as we have seen, can take up in simple solution about 0·87 c.c. of nitrogen for every 100 c.c. Taking the specific gravity of blood as 1·06, we may consider that about 30 c.c. of nitrogen are constantly in solution in the blood. Fat is capable of absorbing six times as much nitrogen as an equal weight of blood, *i.e.* we may write down 500 c.c. as the volume of the gas held by the fatty matter of the body. Leaving out the earthy part of bone, the remaining tissues account for about 435 c.c.

Taking round figures, we see that the average man has, dissolved in his blood, about a litre of nitrogen. The weight of this litre is a function of the pressure under which it has been absorbed. Looked at from another point of view, the weight of nitrogen held in solution by the tissues is 32 times as great as that present in the blood. If, therefore, the blood is, for the purpose of this calculation, considered as spread uniformly and at a uniform rate throughout the body, the tissues would receive at the end of one complete circuit of the blood after exposure to a sudden increase in air pressure, 1/32 of the excess of nitrogen corresponding to complete saturation at the new pressure. The second round of the circulation would add 1/32 of the remaining deficit in saturation, and so on. Haldane finds that it takes 23 rounds of the circulation to half-saturate the tissues at the new partial pressure of nitrogen. The progress of the saturation of the body with nitrogen may be represented by a logarithmic curve (Fig. 84). As about 3·5 litres of blood pass through the lungs every minute, and as the total

blood volume is also 3·5 litres, we may substitute *minutes* for *rounds* of the circulation, and state that it requires 23 minutes to render the tissues half-saturated to a new pressure of nitrogen.

The process of desaturation, provided physiological conditions are kept constant, follows the same curve. If the tissues are exposed to blood-carrying nitrogen in excess of the normal amount, for sufficiently long to be in gaseous equilibrium with that blood—*i.e.* to be saturated—then in order to prevent the formation of bubbles, the process of desaturation would need to be carried out

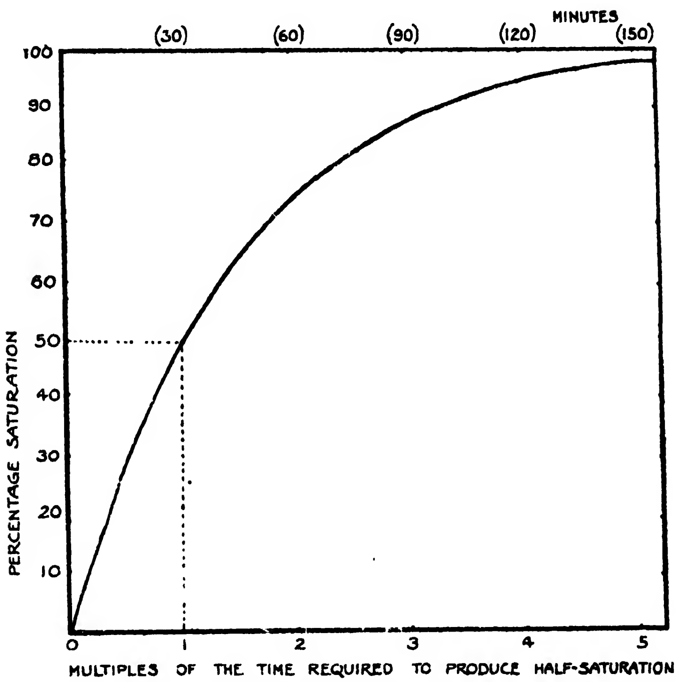


FIG. 84.—Curve showing the progress of saturation of any part of the body with nitrogen after any given sudden rise of air pressure (after Haldane).

at the same rate as the saturation. If the desaturation rate were too rapid, then gas would be released from the tissues more rapidly than it was being passed from blood to alveolar air. This would entail a very slow and uniform rate of decompression. A diver's ascent from the sea bed might have to be spread over hours. Paul Bert, from his experiments on animals, concluded that the decompression period should be 30 minutes for under 3 atmospheres, and 60 minutes for 3 to 4 atmospheres. This ruling of the famous French scientist has never been carried out in industrial practice, the usual period for " leaking out " being about 15 minutes altogether. As a result of this haste to get into

"free air," constructional engineers are afraid to put their men under more than + 3·5 atmospheres. Bullion has been salved from ships lying 171 feet below the surface. The divers in this case stayed below for only 20 minutes at a time and took 20 minutes to ascend. Even then some of them were stricken with paralysis. Greenwood endured compression to 7 atmospheres (= 210 feet of sea water), but took over 2 hours to decompress. These long periods of decompression which seem necessary for safety, put the men in charge in an awkward dilemma when, on account of some mishap, it is necessary to bring men at once to the surface.

From Table LVI. it will be seen that the diver is brought to the surface from the bottom in stages. These stages are 3 metres apart, and the time spent at each one depends on the duration of his stay on the bottom. This method of decompression by stages depends on the empirical fact that no untoward results arise from even a rapid decompression of 1 atmosphere or less.

An atmosphere or 760 mm. of mercury is equal to a pressure of 1 kg. per sq. cm. or to about 3 metres of sea water. Even with this more rapid means of attaining normal pressure, the diver is limited either to a very short stay under water or to a tedious waiting at various levels.

TABLE LVI

A PORTION OF A DIVING TABLE USED BY NAVAL DIVERS

Depth.		Total pressure in atmospheres.	Time from surface to beginning of ascent.	Depth and duration in minutes of stoppages during ascent. Metres.				Time for total ascent in minutes.
Fathoms.	Metres.			12	9	6	3	
18–20	33–36½	4·6	Up to 15 min.	–	2	3	7	15
			15–25 ,,	–	5	5	10	25
			25–35 ,,	–	5	10	15	33
			35–60 ,,	5	10	15	25	57
			60–120 ,,	10	20	30	35	97
			Over 120 ,,	30	35	35	40	192

Haldane and his collaborators have very fully investigated this question. They argue that, as the volume of gas in solution is constant no matter what is the pressure, and as it has been proved to be perfectly safe to decompress rapidly from a plus pressure of 1 atmosphere (1·25 atmospheres to be exact) to normal, then it must be equally safe to decompress rapidly to half pressure for any value. For example, if the total pressure were 8 atmospheres, these workers advise a rapid decompression to 4 atmospheres, and after a pause to 2 atmospheres, and, after

a pause, more slowly to normal pressure. The principle underlying this plan is that the discharge of nitrogen from the start of decompression is at the maximum rate consistent with safety. The rate of discharge, of course, depends on the gradient of pressure between venous blood and alveolar air. This gradient is kept as steep as possible, and there is, therefore, a maximum elimination by the lungs.

CHAPTER XXV

CIRCULATION

> " The circling streams, once thought but pools of blood,
> (Whether life's fuel or the body's food)
> From dark oblivion Harvey's name shall save."
>
> DRYDEN.

THE inland transport system that we have had under consideration differs materially from our canal system. Not only are the barges submersed in the plasma, but the force which carries them along is the force which causes the plasma itself to move. The water-

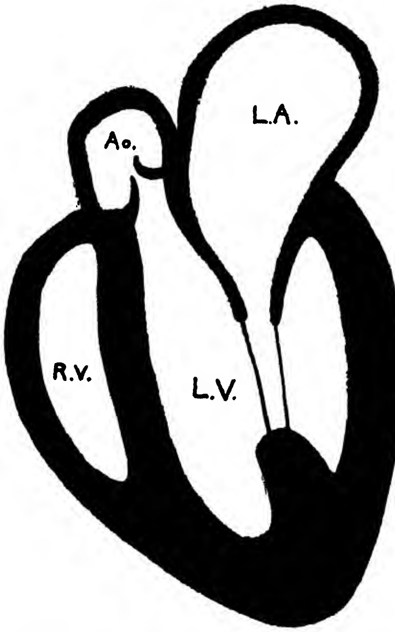


FIG. 85.—Vertical Mesial Section through Heart to show Aortic and Mitral Valves. *R.V.*, right ventricle; *L.V.*, left ventricle with papillary muscle; *L.A.*, left atrium with the mitral valve extending into the left ventricle; *Ao.*, aorta with anterior cusp on top of septum.

(Noël Paton's *Essentials of Human Physiology.*)

ways are a series of elastic-walled tubes forming a closed circuit. In this circuit is a central pumping station, the heart, which keeps the blood in motion. The accompanying figure (Fig. 85) is a diagrammatical view of a vertical-mesial section through the

heart. From it we learn that the heart is not a simple structure. In the diagram four distinct cavities can be seen, viz.: right and left ventricles, left atrium and aortic space—the right atrium is not shown. The heart is really a double pump consisting of a main pump or systemic heart (left atrium and ventricle) and a subsidiary pump or pulmonary heart. In Fig. 86 is given a scheme of the circulation. By contraction of the left ventricle the blood is forced along a series of conducting tubes or arteries (*Art.*) which lead to every part of the body and end in the substance of the tissues in a network of innumerable hair-like canals, the capillaries (*Cap.*). These capillary vessels are the wharves of the tissues. Through their walls takes place the exchange of imports

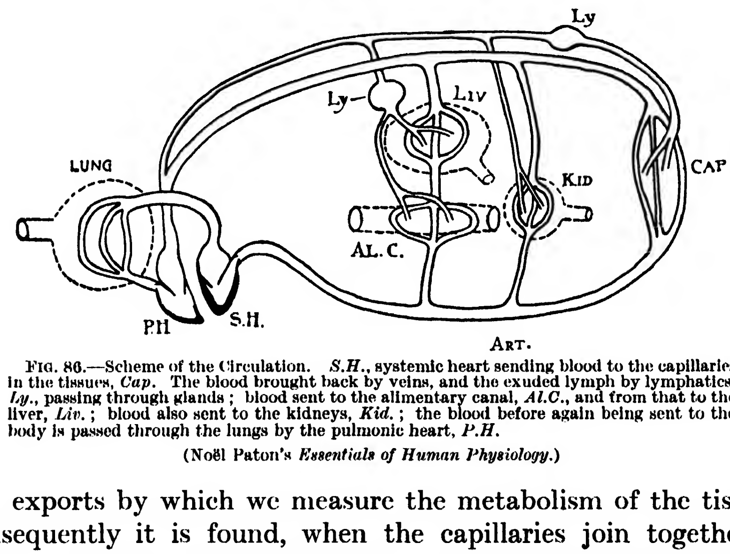


FIG. 86.—Scheme of the Circulation. *S.H.*, systemic heart sending blood to the capillaries in the tissues, *Cap.* The blood brought back by veins, and the exuded lymph by lymphatics, *Ly.*, passing through glands; blood sent to the alimentary canal, *Al.C.*, and from that to the liver, *Liv.*; blood also sent to the kidneys, *Kid.*; the blood before again being sent to the body is passed through the lungs by the pulmonic heart, *P.H.*

(Noël Paton's *Essentials of Human Physiology.*)

and exports by which we measure the metabolism of the tissues. Consequently it is found, when the capillaries join together to form the wider conducting canals, venules and veins, that the blood has lost some of its cargo of oxygen and nutrient matter, and has gained a certain amount of waste matter. This withdrawal of nutrient material is made good by the diversion of some of the blood from an arterial canal to the capillaries in the walls of the intestine (*Al.C.*). Some waste matter is eliminated, as we have seen, by a capillary mechanism in the kidney. Chemical changes occur on the passage of the blood through the capillaries of certain factories, *e.g.* liver and spleen. The loss of oxygen is not made good until the blood has been carried by the veins into the right atrium, passed from this reception house into the body of the pump, the right ventricle (*P.H.*) and forced by the action of this subsidiary pump into the lung capillaries. There, as we saw in the last chapter, it gets rid of the excess of carbon-dioxide

and makes up its deficit of oxygen. Finally the blood, with its fresh supply of nourishing substances from the alimentary canal and of oxygen from the lungs, is poured into the receiving chamber of the main pump—again to pass into the left ventricle and so to the tissues.

From the capillaries some of the constituents of the plasma are forced into the spaces between the cells as lymph. From these spaces the fluid either passes back into the capillaries or flows away in a series of lymph vessels which carry it through lymph glands (*Ly.*) from which it gains certain necessary constituents and finally bring it back to the central pump.

This, in brief, is the circulation as we know it to-day, and this knowledge is due in great part to the labours of Harvey. Before his time little was known of how the blood was distributed in the body. Of one point the old physiologists were sure, and that was that there was no circulation of the blood, only an ebb and flow. Harvey's work is a perfect example of how scientific work should be carried out. First of all, he cleared his mind of all preconceived ideas and got down to bedrock. Then he stated his method. The method employed was that now made famous by the author of Sherlock Holmes, viz., induction, based on careful investigation. He examined the valves of the veins, and using them as sign-posts, traced the course of the blood. Similarly, the valves of the heart permit a current to flow in one direction only. There never was a more complete argument than the one that Harvey pressed for the circulation of the blood. There could be no ebb and flow where all the valves were " one way."

No scientific work is complete without a reference to quantities. The test of truth must rest with the balance or measuring mechanism. Harvey found that the left ventricle of a man's heart held 2 oz. of blood without being distended. If only half the load were discharged at each systole and the heart beat 70 times per minute, then 700 oz. or 44 pints of blood would be discharged into the aorta every 10 minutes. The total blood volume is under 9 pints. From this he argued the necessity of some communication between the arteries and veins. That is, after experiment, observation, analysis and argument, come reasoned hypotheses. Four years after Harvey's death the great Italian anatomist Malpighi saw under the microscope these capillaries which the physician had seen with the eye of faith. The demonstration of the actual passage of blood from arteries to veins through capillary channels was given in 1688 by Leeuwenhoek, the illiterate janitor of the aldermen of Delft.

Haemodynamics. Dynamically considered, the blood acts in

much the same way as any other equally viscous fluid driven through a series of tubes. In order to understand many of the problems which one meets in the study of physiological phenomena, it is necessary to obtain some insight into the movement of fluid under an external driving force. As Servetus says, "In order to learn how the blood is formed it is necessary to ascertain how it moves." First of all, let us consider the flow of liquid from a reservoir through a series of tubes.

(1) **Gravity.** In a liquid the molecular forces are in equilibrium; the kinetic forces characteristic of matter in the gaseous state are exactly balanced by the Newtonian forces predominant in solids. As Soddy would put it, the processes of *pellation* and *tractation* would not be manifest. Gravitation alone has to be reckoned with. In common parlance, liquids seek their own level and so always tend to flow to the lowest possible position. It is a well-known fact that the speed attained by a body falling *in vacuo* through the distance (h) equals $\sqrt{2gh}$, g being the acceleration produced by gravity.

(2) **Resistance at Outlet.** This formula cannot be used to estimate the velocity of fluid escaping from a reservoir. As every boy knows, when the waste water is being run out from the bottom of a wash-hand basin, the fluid tends to rotate round the orifice and to assume a conical form. This is due to the attempt of the water particles to rush the exit (so to speak). Only a limited number of them lie in the column vertically above the opening. The majority, occupying more lateral positions, tend to escape along with the minority in the queue and so exert a force applied at an angle to the line of exit. Consequently, the total energy cannot be used to produce velocity. *Some of it has to be spent in overcoming the resistance at the outlet.*

(3) **Resistance to Flow.** Still further modification of the formula is required if the orifice is fitted with an exit tube. It must be evident that the presence of this passage imposes a greater resistance to outflow and materially reduces the rate. Let us consider the effect produced on rate of flow by attaching a rigid cylindrical tube of uniform bore to the lower orifice of the reservoir. In order to simplify matters, we will place this pipe horizontally. Two causes tend to reduce the kinetic energy of fluid flowing through a tube, viscosity or internal friction and external friction on the walls of the tube.

(*a*) *Friction.* On account of the latter, the outermost layers of the fluid adhere to the walls of the tube and become more or less stationary. The molecules of the layers of fluid next to the outermost tend to cohere to the stationary layer on one hand and are

pulled along by their cohesion to the next inner layer. As a result, their velocity is decreased. The net result is that a whole series of cylindrical layers is produced each with a different rate of flow—ranging from the almost stationary outer layer to the central axial column, which is retarded least of all and, therefore, possesses the greatest kinetic energy. In a straight tube of uniform bore, such as is under consideration, this retarding influence reduces the average rate of flow to half that of the axial stream. It is obvious, therefore, that a considerable amount of the potential energy of the liquid in the reservoir is absorbed in overcoming the peripheral resistance caused by pure friction. ***Resistance to flow also depends on the area of cross-section of the tube***—the wider the tube the larger the number of cylindrical layers over which the adhesive resistance spends itself and, therefore, the less the resistance met by the axial stream. Liquid, in a tube so narrow that only an outer layer and a central column could pass along it, would move with infinite slowness. Except in instances in which the conducting tube has a very large or a very small diameter, the rate of flow is proportional to the area of cross-section. *Further, the resistance in a tube of uniform diameter is proportional to its length.* Therefore, the energy of the fluid must decrease gradually from the reservoir to the outlet of the tube.

(*b*) *Viscosity.* The internal friction or viscosity depends on the nature of the fluid, and, as indicated in Table XLIII., on the size and concentration of the bodies suspended in it. The mean viscosity of blood compared with water is 4·45, and, therefore, it would require 4·45 times as much pressure to force blood along a tube at the same rate as an equal volume of water. The blood cells do not materially affect viscosity till they occupy about two-thirds of the total volume, *i.e.* till the haematocrite reading is about 66 per cent. When that concentration has been reached, the value of the viscosity rises owing to the friction of one erythrocyte against another. The values of the viscosity of blood vary at different parts of the circulation, due principally to alterations in the size of the corpuscles (*q.v.*). The total frictional loss (friction + viscosity) reaches a maximum in the capillaries, where not only do the corpuscles tend to increase in volume but the bore of the individual vessel becomes so small that the corpuscles undergo considerable distortion in their passage along it.

Pressure. The energy of the fluid is shown by the pressure it exerts. Pressure may be measured by some form of manometer. It is sufficient to insert a number of vertical glass tubes, of uniform bore and open to the air, at various points of the conducting tube to see the fall of pressure with distance from the source of power.

The fluid rises in these collateral tubes or *piezometers* to a height proportional to the pressure in the main conduit. In other words, the level of the liquid in those pressure-gauges is accurately adjusted to the peripheral resistance encountered by the liquid as it passes their points of insertion. Such a system is represented in Fig. 87. The power furnished by the liquid, in the constant-level reservoir (**R**), is the downward pressure of gravity. The pressure at various points is manifested by the height of the fluid in the branch tubes (*A*) 1, 2, 3, etc. If the levels of the column of liquid in each of these piezometers be joined by a straight line which is produced to the reservoir wall at (**y**), the mass of liquid will be divided into two portions. The lower portion (**r**) represents the portion of the energy of the total spent in overcoming the

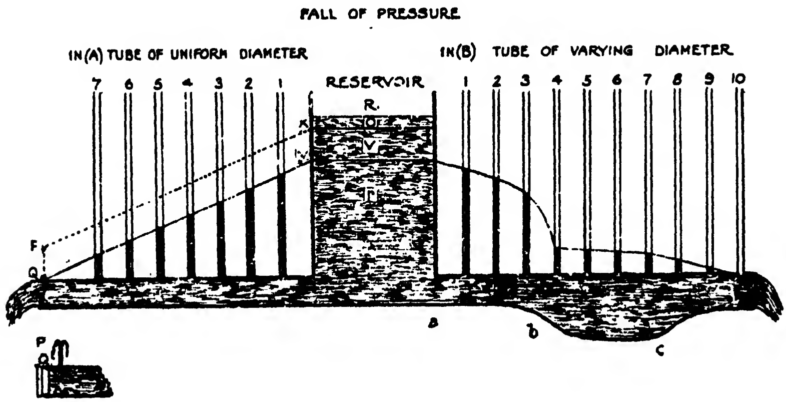


FIG. 87.

resistance, and is consequently known as *resistance-pressure.* Of the remainder, a certain amount (**o**) is spent in forcing the fluid through the orifice into the tube. The actual driving force or velocity pressure comes from the mass (**v**).

If the main tube is not of uniform bore—suppose (*B*) it increases in sectional area, at first gradually (**a** to **b**) and then somewhat suddenly (at **b**)—corresponding alterations in pressure may be seen in the manometers. Increase in width means smaller resistance, and therefore a smaller resistance-pressure is required to drive the fluid along the tube. As the total mass in the reservoir is kept constant, the amount not required in **r** goes to increase **v**. There being relatively a greater head of pressure, the levels shown by the manometers will tend to decrease progressively at a slower rate than before. If, on the contrary, the bore of the tube is diminished as at **c**, the fall of pressure will become more rapid. Further, if at **b** a constriction is produced, resistance to flow is

augmented, and therefore there is a heaping up of the fluid in the earlier tubes 1, 2 and 3, a rapid fall to tube 4, and thereafter a fall of pressure at *the same rate* as in the earlier part of the system. All the above is stated in terms of pressure. Putting the same matter in terms of velocity of flow, one may say that if a tube be used, the second segment of which is wider than the first and third, the speed of flow will be decreased in the central one.

Pressure by Force-Pump. In the preceding experiment, the

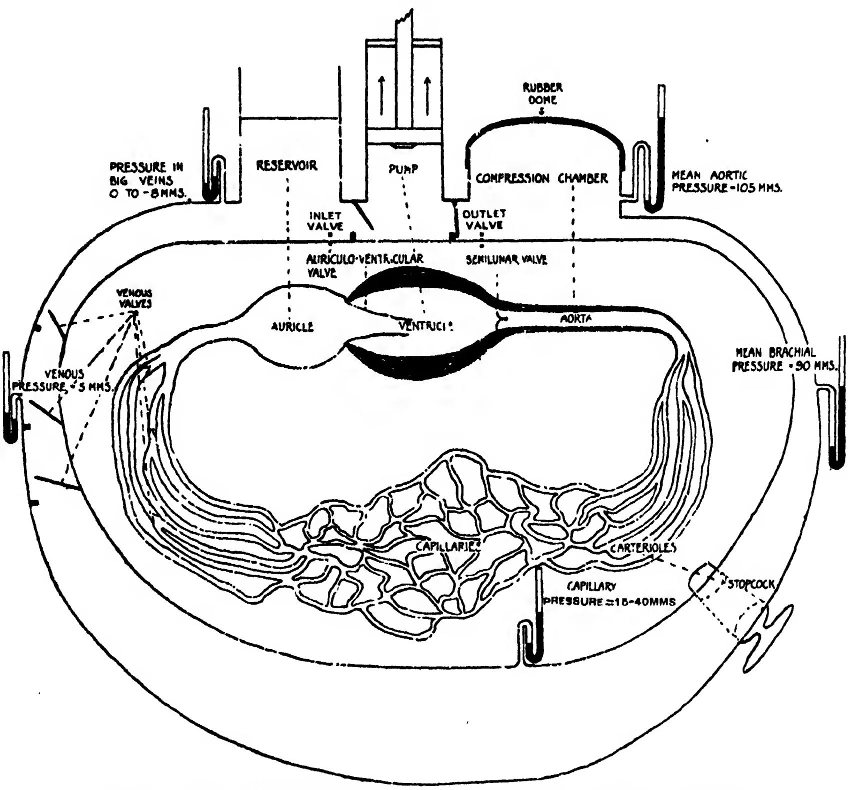


FIG. 88.—Diagram of a simple force pump (outer circuit) to compare with diagram of circulation from left side of heart (inner circuit).

head pressure has always been kept constant by making provision for a steady influx of water to the reservoir to compensate for the outflow. If, however, the head of pressure is produced by the action of a piston in a cylinder, it will not cause a continuous but an intermittent flow in the main conduit. The pressures shown in the piezometers will vary from a maximum to a minimum as the wave of pressure passes down the system after each stroke. Such conditions entail great loss of power.

Elastic Regulator. In order to reduce this loss to a minimum,

it is necessary to replace the rigid conduit by an elastic tube. Such a tube would, of course, if rigid, permit a certain flow of fluid per unit of time per unit of pressure, say with a constant velocity of (**v**). Now on the descent of the piston more water tends to be forced into the conduit than can be passed out with this velocity (**v**). The elastic walls distend till their elastic power exactly counterbalances the extra energy, and the fluid has an outflow velocity of (**v**). The influx of water having ceased, the steady pressure of the distended walls of the tube as they recoil keeps the fluid at the constant velocity (**v**). In this way the fluid is held under a continuous pressure, and, provided the pump has the proper frequency, the outflow remains practically constant. That is, *the elastic tube really converts an intermittent inflow into a constant outflow, the property of elasticity preserving normal conditions of flow even during periods when the piston is not descending.*

The value of **the work done** by a pump may be calculated approximately by the formula

$$W = QR + \frac{mv^2}{2g} + O,$$

where **W** (gram-metres), is the work done at each stroke, **m** is the mass in grams and **Q** the stroke volume, the quantity of fluid in c.c. expelled at each stroke; **R** is the average resistance of the circuit, **v** (metres per sec.) is the velocity of expulsion, and **g** is the acceleration due to gravity = 9.8 metres per sec. per sec. That is, **QR** represents the resistance pressure (**r** in Fig. 87) and $\frac{mv^2}{2g}$ (**v** in Fig. 87) the velocity pressure, while **O** is the energy expended in overcoming the resistance to outflow at the orifice of the pump.

Such a system of single stroke pump and elastic regulator does not differ in essentials from the one contrived by nature to provide a perfect transport service to every unit of a complex organism like the human body. In Fig. 88 a simple force pump and its circulating system is compared with the left ventricle, aorta, etc.

The manner in which the contents are forced out from the ventricle differs in some details from that obtaining in the water pump. In the latter, a rigid piston descends within a rigid cylinder and thus obliterates the space of the main chamber and forces the water through the outflow pipe. The power necessary to drive the plunger home is derived from an engine of some sort, external to and independent of the pump itself. In the heart, the elastic muscular walls of the ventricles contract as a whole,

deriving their force, just as any other muscular structure does, from the potential energy of materials brought to them by the blood and liberated in their protoplasm.

Work done by the Heart

If we take average figures for the human left heart as follows:

$$\begin{aligned} Q &= 60 \text{ c.c.,} \\ R &= 100 \text{ mm. Hg pressure in aorta} \\ &= 0{\cdot}1 \times 13{\cdot}6 \text{ grams (1 c.c. of Hg weighs } 13{\cdot}6 \text{ gm.),} \end{aligned}$$

the expression QR may be evaluated as

$$60 \times 0{\cdot}1 \text{ m.} \times 13{\cdot}6 = 81{\cdot}6 \text{ gram-metres.}$$

That is, about 80 gram-metres of work is done in overcoming the resistance of the conducting tubes. This value is only approximate, as the work done in forcing a fluid along an elastic tube in which the pressure falls steadily, say from 150 mm. Hg to 50 mm. Hg is not strictly proportional to the average pressure, but would need to be determined by integration. The error is, however, less than 10 per cent. If the blood is expelled at a velocity of 0·4 metre per second, the velocity pressure will have a value

$$\frac{mv^2}{2g} = \frac{60 \times (0{\cdot}4)^2}{2 \times 9{\cdot}8} = 0{\cdot}5 \text{ gram-metre.}$$

This quantity is so small compared with the former during rest that for all practical purposes the work of the heart may be taken as proportional to the *output* multiplied by the *average arterial pressure, i.e.* $W = Q \,.\, R$.

Similarly the work of the right heart may be estimated from the average pressure of the pulmonary artery (20 mm. Hg) as $60 \times 0{\cdot}02 \times 13{\cdot}6 = 16{\cdot}1$, say 16 gram-metres per beat. The average heart beats 70 times per minute, and, therefore, in 24 hours the work done by the heart (of a man at rest) will be about 10,000 kilogram-metres.

Muscular work, of course, augments this figure not only by increasing the volume of blood per beat and increasing the number of beats but by raising the value of the velocity factor. When the output is increased to 20 litres per minute, as it may easily be during exercise, as is shown in Table LVIII., the velocity factor becomes about 10 per cent. of the total work of the heart and must be taken into account. The following table (LVII.), taken from Lovatt Evans, indicates the variation in the magnitude of the velocity factor with the output of the heart.

TABLE LVII

OUTPUT AND MEAN AORTIC VELOCITY OF BLOOD FROM THE DOG'S HEART (Lovatt Evans.)

Output (Litres per hour).	Mean Aortic Velocity (Metres per second).	Kinetic Factor for both Ventricles * (in kg. metres per hour).
3	0·042	0·0038
12	0·169	0·253
48	0·67	15·4
96	1·35	125·0
120	1·69	245·0

During a short sprint an athlete may have a pulse rate of 180 per minute with an output of 180 c.c. at each beat and an average arterial pressure of 120 mm. Hg. Then for the systemic heart:

$$QR = 180 \times 0{\cdot}12 \times 13{\cdot}6 = 294 \text{ gram-metres.}$$

and $$QR \text{ (pulmonary)} = 180 \times 0{\cdot}025 \times 13{\cdot}6 = 61 \text{ gram-metres.}$$

If the time of outflow is considered as three-eighths of each cardiac cycle, of which there are 180 per minute, then the contents of the ventricle, 180 c.c., are shot into the aorta at the rate of 32·4 litres per minute. If the cross-section of the root of the aorta be taken as 625 sq. mm., then the mean aortic velocity will be 540/625 = 0·86 metres per second. Now, as blood is expelled only during a period not greater than about three-eighths of the cardiac cycle, the average velocity of expulsion must be, *at least*, 8/3 times as much as the mean aortic velocity; hence

$$0{\cdot}86 \times 8/3 = 2{\cdot}3 \text{ metres per second.}$$

Therefore, $wv^2/2g = 180 \times (2{\cdot}3)^2/2 \times 9{\cdot}8 = 48{\cdot}8$ gram-metres.

That is, the total work on both sides of the heart will be

	Left side.		Right side.	Total.
During rest	(81·6 + 0·5)	+	(16·1 + 0·5)	= 98·7 gram-metres.
During work	(294 + 48·8)	+	(61 + 48·8)	= 452·6 gram-metres per beat.
				= 81·5 kilogram-metres per minute.

The main fault to be found with this calculation is that the various quantities required are almost impossible to obtain. For instance, the only methods by which the output of the heart *in situ* can be determined are indirect. Zuntz, by finding the

* Calculated from Lovatt Evans' formula $= \dfrac{7mv^2}{g}$

percentage amount of oxygen which the blood gains per unit of time in passing through the lungs, and the actual amount of oxygen taken from the lungs per unit of time, calculated the amount of blood that had passed through the lungs during that period. For example, if the blood gains 5 per cent. of oxygen and the lungs part with 30 c.c. of oxygen to the blood, then, in order to have a 5 per cent. mixture, 600 c.c. of blood must have passed through the lung in unit time. Now if the heart beats 70 times per minute, and the unit of time chosen was one-fifth of a minute, then the volume of the right ventricle would be

$$5 \times 600/70 = \text{almost } 43 \text{ c.c.}$$

Since, of course, the left and right ventricles must each discharge equal amounts of blood, the output of the left ventricle is found.

Total Work of the Heart

Lovatt Evans has shown that if the pressure in the right ventricle be assumed to be one-sixth that of the left, a close approximation to the total work of the heart can be obtained from the expression :

$$W = \frac{7QR}{6} + \frac{m(VC)^2}{gE^2}$$

where E = duration of period of expulsion,
C = duration of cardiac cycle, and
V = mean aortic velocity.

Stroke Volume. Muscular work causes an increase in the output per beat. Under resting conditions, it is probable that the amount of blood entering the heart during the diastole is not sufficient to fill the ventricle up to the limits set by the fibrous inextensible bag surrounding the organ (pericardium). The first effect of the call for more oxygen set up by the muscles is to increase the output of the heart per beat. The power of the heart thus to increase its

TABLE LVIII

Effect of Work on Cardiac Output

Muscular work per min. in Kgms.		Pulse rate per min.	Output per beat in c.c.	Output per min. in c.c.
At rest . .	0	70	45	3,150
Moderate exercise .	270	100	75	7,500
		110	120	13,200
Light labour .	735	130	115	14,950
Very hard work .	1000	180	117	21,060

capacity is limited. By a reflex mechanism the heart rate is increased and so the output per minute is augmented. The table on p. 370 shows approximately the share of the burden of increasing the output borne by increased distension of the ventricular walls and by increased pulse rate.

It will be seen that at first the pulse rate is practically unaltered although the amount of work done has been increased from 270 to 735 kilogram-metres while the output per beat has increased from 75 to 120 c.c. After this, the output per beat is not materially changed, if anything it tends to decrease, while there is a marked increase in the pulse rate. It is interesting to note the increase in the rhythm of the heart when work has just been started, viz. from 70 to 100. This is associated with the initial changes originated by the acts of volition and attention. The mere caution, "Are you ready?" is sufficient to cause a rise in the pulse rate due, in part, to the increase of muscular tone in the act of attention, and, in part, to psychological causes.

A fair day's muscular work may be taken at 100,000 kilogram-metres. We have seen that the work done by the heart is, at least, 10,000 kilogram-metres per day. Hence the work done by the heart is always more than one-tenth of that done by the skeletal muscles.

Efficiency of the Heart under Various Conditions

The efficiency of the heart may be taken as the percentage amount of the energy taken in as fuel that is converted into work. Workers in this field are agreed that it is extremely probable that the sole normal source of cardiac energy is the glucose taken to the heart by the blood and in part stored as glycogen in the heart substance. This storage of glycogen renders difficult the interpretation of the results of estimations of the amount of glucose in the blood before and after passing through the coronary vessels. More accurate calculations of the energy generated during the cardiac cycle can be made from the oxygen consumption and carbon dioxide production during bodily rest and during measured work. The table on p. 372 from a paper by Evans and Matsuoka demonstrates this method for obtaining a value for the efficiency of the heart. The total output of blood from the ventricle is fairly constant—averaging about 16 litres per hour. The resistance to outflow was increased by steps of 40 mm. Hg from 80 to 160 mm. Hg, corresponding to an increase in cardiac work of about 10 kilogram-metres a time.

To free the energy necessary for this increased work the heart uses up more oxygen. The amount of oxygen (in cubic centimetres)

TABLE LIX

EFFICIENCY OF THE HEART UNDER VARIOUS CONDITIONS

	Pulse-rate.	Arterial pressure in mm. Hg.	Total output per hr. Litres.	O_2 used per hour in c.c.	Energy generated in Kg. metres.	Work of heart in Kgs. per hr.	Per cent. Efficiency.	Venous pressure. mm. H_2O.
A	134	80	17·4	139	288	21·1	7·3	38
	—	120	16·3	175	362	30·7	8·5	50
	150	160	14·7	249	518	40·8	7·9	80–103
B	143	80	27·8	217	448	34·1	7·6	
	—	80	52	277	572	65·6	11·5	
	146	80	92	649	1,343	126·3	9·4	

so used multiplied by 2·07 gives, in kilogram-metres, the energy developed. It is clear that, with a moderate increase in arterial resistance, the mechanical efficiency of the heart improves, but tends to decrease when the resistance is doubled. In other words, when the arterial pressure is raised, the oxygen intake is increased, and more tension developed in the cardiac muscle. The mechanical efficiency is raised to a certain limit, beyond which it again diminishes. The venous pressure in the experiment quoted, and in most others, runs parallel with the oxygen usage. In the series of observations tabulated as *B*, the arterial pressure was kept constant at about 80 mm. Hg, while the output per hour was increased roughly as 1 : 2 : 3. This was done by varying the inflow of blood to the heart. The increase in oxygen usage is not quite proportional to the increase in work done, but is, if anything, less. The efficiency values, therefore, tend to increase with increasing outputs up to a certain limit. Beyond this point, the amount of oxygen used increases very suddenly. In the example given, for a little less than double the output, almost two and a half times as much oxygen is required. As this involves the liberation of enough energy to lift 1,343 kg. to the height of a metre, and as only 126·3 kilogram-metres of work are done, the increased work is not done so economically and therefore the efficiency value falls.

Maximal Efficiency. How can this primary increase in efficiency and subsequent decrease be explained, and what factors are brought into play to settle the critical point at which maximal efficiency will be found ? If output is to be increased, intake must first be increased and the ventricle must be distended to hold the extra amount of blood. That is, the muscle fibres of the ventricular wall will be stretched. We have already mentioned, in connection with skeletal muscle, that a stretched muscle develops more tension

during the isometric phase. The heart responds to increased work by such a lengthening of its fibres. If the lengthening process is carried too far, the muscle fibres per unit of area will become fewer, so that the larger the ventricular volume, the more strongly will each fibre have to contract in order to produce a given tension. At this greater length they also use up more potential energy just as skeletal muscle does.

We have seen (Chap. XIV.) that when skeletal muscle contracts about two-fifths of the energy used is actually converted into tension. If all the tension energy were then converted into external work, the mechanical efficiency of this type of muscle would be about 40 per cent. The realisable efficiency differs from this theoretical value, because, even provided the load and rate are optimal (*q.v.*), a considerable amount of energy is rendered unavailable for work because it is dissipated in overcoming the resistance of the viscous muscle to shortening. The more rapid and the more complete the shortening, the greater will be the amount of energy lost. The optimum efficiency is obtained when the muscle pulls on a load that is always optimal, *i.e.* varies so as to be always as great as the muscle can move. At the beginning of contraction the load should be great, and it should gradually be decreased as the shortening process proceeds.

This desideratum is found in the heart. As soon as the ventricles start to empty, the shortening cardiac muscles have a steadily decreasing mass of blood to act against.

It is of further interest to note that during severe muscular exercise optimal conditions are found for cardiac efficiency, *i.e.* a high output at moderate arterial pressure. Under these circumstances the efficiency of the heart is about 26 per cent. (cf. Muscle).

Form and Function

Pressure Developed in Ventricles. The diagrammatic section of the heart (Fig. 85) demonstrates that the walls of the left ventricle are much thicker than those of the right. The mean of a large number of determinations furnishes the ratio of 6·8 : 1. This may be interpreted as indicating that the left ventricle develops six to seven times as much pressure as the right ventricle. Proof confirmatory of this deduction is obtained by determining the hydrostatic pressures necessary completely and symmetrically to fill these two chambers. The right ventricle is dilated by a seventh of the pressure employed in equally dilating the other ventricle.

A dog weighing 10 kilos with an average aortic pressure of 100 mm. Hg, and an output of 2,000 c.c. of blood per minute, develops pressure in right and left ventricles of 25 and 150 mm. Hg respectively—a ratio of 25 : 150 = 1 : 6.

The pressure developed in a distended hollow elastic vessel depends on (i.) the elasticity of the walls, (ii.) the degree of distension, and (iii.) inversely, the radius of curvature of the walls. The volume output from both ventricles is the same and their radii of curvature are similar. There remains only a marked difference in elasticity. As both are formed from the same material, alteration in elasticity must be brought about by alteration in wall thickness.

Sections of the ventricles at different points show that the ventricular walls vary in thickness at different parts. For instance, in the left ventricle the apex, in the fully dilated ventricle, has, by far, the thinnest wall. As presumably the pressure in the chamber is constant over the whole wall area at any moment, some other factor must be found to account for this diminution in thickness. From the purely physical study of the shape assumed by elastic-walled cavities the conclusion has been drawn that where an elastic membrane is subjected to internal pressure, its shape will be determined by the law of distribution of radial pressure. With a given shape and size of body, equilibrium is maintained by altering the thickness (resistance to pressure) of the wall so that *where curvature is least the wall is thickest* and *vice versâ*. The apex of the heart is the portion with the greatest curvature.

To take a very simple example: if an elastic band is stretched between two points on a flat surface it will exert no pressure on any part of the underlying surface. But if it is stretched over a curved surface, *e.g.* a cylinder, it will exercise a downward pressure depending on the radius of the cylinder. A flat surface may be considered as equivalent to a curved surface of infinite radius. As the numerical value of the radius is decreased, *i.e.* as the curvature is increased, the pressure exerted by the band will increase. In mathematical form $p = T/R$, *i.e.* Pressure per unit of surface = Tension of band divided by Radius of curvature.

Where there are curvatures in two dimensions, *e.g.* a sphere, the two pressure effects are additive, *i.e.* $p = \frac{2T}{R}$.

The ventricles are roughly egg-shaped, *i.e.* they have radii in two dimensions and of unequal length. The pressure will therefore be equal to the sum of these, *i.e.* $p = T/R + T/R_1$.

We have seen reason to correlate thickness with pressure.

We may therefore say that thickness of wall varies inversely with the radius of curvature. This gives the formula

$$t(1/R + 1/R_1) = C,$$

where t = thickness of the walls and C a constant.

The wall of the apex of the heart has the largest mean curvature (R is least and, therefore, t is least).

Similar reasoning may be applied to the consideration of the thickness of the walls of the blood vessels. The pressure (P) within the vessel is balanced by (1) the elastic tension of the wall (T) divided by the radius of curvature (R), and (2) by the pressure brought to bear on the external surface of the wall by the resistance to distortion of the surrounding tissues (p).

Thus $$T = R(P - p),$$

or putting $t =$ thickness and $C =$ a constant, we may write

$$t = CR(P - p).$$

That is, if $(P - p)$ be kept constant the thickness of the walls will vary as the radius of curvature.

TABLE LX

THICKNESS OF WALLS AND DIAMETER OF LUMEN OF ARTERIES IN MM.

Artery.	Intima.	Media.	Adventitia.	Total.	Lumen.
Brachial (human) .	0·03	0·5	0·25	0·78	4·17
Carotid (ox) . .	0·84	1·176	0·484	1·744	6·0
,, (sheep) . .	0·028	0·420	0·168	0·616	3·0
Metacarpal (horse) .	0·03	0·513	0·31	0·853	2·7

(MacWilliam and Kesson.)

According to measurements made on excised vessels the carotid artery of the ox has a lumen of 6 mm. while that of the sheep is 3 mm. The maximal pressure developed in these vessels at body temperature amounted to 60 and 40 mm. Hg respectively.

That is $R/R_1 = 2/1$ and $p/p_1 = 3/2$.

Now $t/t_1 = R/R_1$ by $p/p_1 = 6/2 = 3/1$.

The actual thickness of the carotids as measured by MacWilliam and Kesson are 1·74 and 0·61 mm. respectively. Of course, the main elastic resistance to distortion is met with in the muscular *tunica media*, which in the ox is 1·12 mm. and in the sheep 0·42 wide. Either pair of figures gives a close approximation to the ratio 3 to 1 (Table LX.).

Valves.

The valves of the heart and veins are interesting mechanical structures. During the two years that Harvey studied at the University of Padua, Fabricius, the renowned Professor of Anatomy there, was investigating the valves of the veins. He demonstrated their presence in the veins of the arms and legs and also in the

vessels at the root of the neck. These sluice-gates are very simple contrivances—just little pockets set in pairs opposite each other in the vein. Fabricius noted that the openings of the pockets were always directed toward the central part of the body. He interpreted this as indicating a mechanism to prevent the blood from gathering, under the influence of gravity, in the lower parts of the body. Harvey saw that this explanation did not account for the setting of the valves in the veins of the neck, and, by noting the direction in which the valves would allow fluid to pass, he discovered the circulation of the blood.

It is clear that the pockets offer practically no resistance to the passage of blood towards the heart. If, however, the pressure on the heart (or central) side of a valve becomes greater than the pressure in the preceding segment, the pockets will fill with blood, become distended and effectively prevent a back-flow. That this is so can be proved by repeating one of Harvey's experiments. He tied a ligature round the upper part of his arm and so dammed up the blood in the lower part of the arm. When he milked these swollen veins towards the hand he noticed that the blood could not pass certain points where he knew valves were placed. No valves are necessary in the arteries as there is always a positive driving pressure. The type of two of the valves of the heart is indicated in Figs. 85 and 88.

(1) **The atrio-ventricular valves** are triangular sheets of fibrous tissue—tough but flexible—fixed by one side to the atrio-ventricular ring and hanging apex downwards into the ventricular cavity. The pointed part of each flap or cusp is tied to the ventricular wall by a number of cords, *chordae tendineae*. The main cords are, however, not inserted directly into the ventricular wall, but are attached to the finger-like papillary muscles. These muscles regulate the tension of the valve-flaps. The bases of the valve-flaps are approximated by the ventricular contraction which begins at the base. When the ventricles contract so do the papillary muscles—pulling on the *chordae* and thus preventing the cusps from being pushed through into the atria. The increasing pressure of the blood in the ventricle causes the flaps to belly out and block the passage-way so that the blood cannot pass back into the atria. The greater the pressure developed in the ventricle, the more tightly is the valve shut. The cusps may even bulge up into the atria. Valves constructed on this principle are obviously fitted to occlude openings which vary in size and shape during the various phases of the cardiac cycle.

The right and left sides of the heart differ in the number of cusps in their valves and in the details of their movements. The

mitral valve on the left side of the heart has only two triangular flaps like a bishop's mitre, while, on the other hand, the passage way from right atrium to right ventricle is guarded by the three cusps of the **tricuspid valve.**

During systole, the strong anterior cusp of the mitral valve does not materially shift its position. The other cusp is pulled forward against it.

On the right side, one of the cusps hangs down on the septum and is practically immovable. The other two cusps are pulled over towards the septal cusp. The mass of blood pressing on the sides of the cusps completes the closing of the orifice.

When this mass of blood, under the pressure induced by the

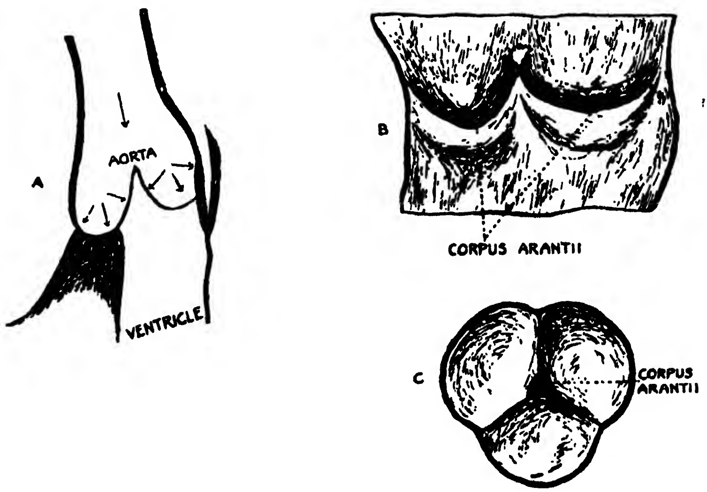


FIG. 89.—Semilunar valves. *A*, in longitudinal-mesial section. *B*, Artery laid open and exposed, and *C*, closed valves from the arterial aspect.

contraction of the ventricles, stretches the atrio-ventricular valves it causes them to emit a sound which is a component of the first sound of the heart. The other component is the sound produced at the same time by the contraction of the ventricular walls. It is said that a trained ear can pick out the notes due to closure of the valves from those due to stretching of the muscular walls.

(2) Semilunar Valves. The valves situated at the openings of the ventricles into the arteries are similar in shape and in action to the pocket valves of the veins (Fig. 89). Each is composed of three pockets or half cups attached along their curved margins to the walls of the artery and upper part of the ventricle and with their openings set away from the ventricle.

The cusps are not placed all exactly on the same plane. One cusp lies somewhat deeper in the heart than the others. This

cusp is mounted on a muscular septum which acts as a cushion, absorbing the shock when the pressure falls on the valve and the other two cusps shut down on it.

The sudden stretching of these **semilunar** valves by the impact of the high arterial pressure sets the valves in vibration like the blow of a drum-stick on a drum-head. It produces a clear, sharp, high-pitched sound, the so-called **second sound of the heart.**

A **third sound** has been described. It has been attributed to the rebound of the atrio-ventricular valves when the ventricle relaxes and the atrio-ventricular orifice again becomes patent.

When the valves are diseased certain more or less continuous sounds or **murmurs** are heard. They are in the main due to either of two causes.

(1) **Stenosis.** When a fluid flows along a tube of uniform bore or a tube where the bore alters gradually no vibrations are set up. On the other hand, if the cross-section is altered suddenly and appreciably, the fluid is set into vibrations. These vibrations are transmitted to the solid tube and to the material in which it is set and a sound is produced. Most people have heard the rather irritating *purr* emitted by the domestic water supply when there is " air in the pipe." The vibrations may not only be heard but they may be felt at the tap and seen in the water issuing. Something similar takes place when, by disease, the opening from atrium to ventricle is narrowed. During the whole period when the ventricle is filling up from its atrial reservoir, the blood flowing through the narrowed opening is set into vibrations which are transmitted through the more solid tissues to the inner ear—this is the murmur of mitral or of tricuspid stenosis, according to whether the fault lies on the systemic or pulmonary side respectively. The narrowing does not need to be absolute. If the previous part is dilated, *the orifice will become relatively narrower* and will produce the result.

Similarly the murmur caused by stenosis of the aortic or of the pulmonary valves will be heard during the expulsion of blood from the ventricles.

The effect of the narrowing of the aortic orifice on the magnitude of the velocity component of cardiac work is considerable. If the orifice be decreased in area to 1 cm.^{2}, for instance, the velocity of the blood in passing this very narrow orifice is so much increased above the normal value of 0·4 metre per second that, in spite of the decrease in heart rate characteristic of this condition, the velocity component may reach a value of about half that of the total work of the heart. An enormous hypertrophy of the left ventricle is,

therefore, produced to allow of this extra work being done. In a case like this, it is not surprising to find later that the heart is unable to respond to the call for any extra effort, and that even slight exercise results in distress.

(2) **Incompetence.** The failure of any of the valves to close completely allows blood to trickle back into the empty expelling chamber. This regurgitation throws the tightly stretched cusps into vibration and produces a murmur. If this sound is heard during ventricular systole it may be ascribed to incompetence of either of the atrio-ventricular valves—if during ventricular diastole, the aortic or pulmonary valves are at fault.

In aortic incompetence the sound will be best heard where the aorta comes nearest to the surface, viz. at the second right costal cartilage; in pulmonary incompetence the murmur will be best heard over the second left interspace just external to the margin of the sternum.

The sound of the mitral valve is heard at its best just over the apex of the heart; that of the tricuspid valve at the junction of the fourth right costal cartilage with the sternum.

By means of a recording microphone, a tracing may be obtained representing the values of these sound waves. Such a phono-cardiogram (Fig. 93, Chap. XXVI.), if taken simultaneously with a tracing of the mechanical or electrical changes of the heart, is of great use to the physician as an indication of cardiac efficiency.

If any of the large arteries be compressed, say by the imposition on the overlying skin of the stethoscope, murmurs will be heard. These sounds are caused by the sudden narrowing of the lumen of the artery by the pressure of the instrument. The blood rushes through the narrowed part into the comparatively wide part of the vessel beyond the point of pressure and so sets up eddies. The vibratory movement of the fluid is transmitted to the arterial walls and passed on to the internal ear (Part II.).

Considering the circulatory mechanism as a whole, one is struck by the extraordinary efficiency of this method of transport. Comparatively little energy is wasted. Fluid leaves the ventricle under a pressure of over 100 mm. Hg, passes through a system of large and small tubes and returns to the reservoir of the central pump with *no surplus pressure.* Just enough blood pressure is provided to carry the fluid within range of the atrial suction and no more. It has been stated that by the rhythmic contractions (peristaltic waves) of the muscular coat of the vessels, the blood is helped along its course. The mechanics of peristalsis will be considered shortly (Chap. XXVIII.).

Angle of Origin of Vessels

One further point making for the economical working of the inland transport service, owes its enunciation to John Hunter. He wrote, "To keep up a circulation sufficient for the part and no more, Nature has varied the angle of the origin of the arteries accordingly." Suppose a point C is h units vertically distant from an artery AB, the problem is to find out the route by which the blood could be conveyed from A to C with the least possible loss of energy. This is not necessarily by the shortest route or by the route using the shortest piece of branch tubing. The shortest route would be h units long and would arise from AB at right angles (say at D). For the purposes of this calculation let us consider that the least loss of power occurs when the branch originates at X which is x units from D, making an angle of θ with the main trunk. Then the distance from X to C would be $\sqrt{x^2 + h^2}$ (hypotenuse of right-angled triangle).

Assuming that loss of pressure is due to friction on the walls of the vessels, then it will be directly proportional to their lengths and indirectly proportional to their radii (*e.g.* main trunk $= R$ branch $= r$);

i.e. loss is proportional to $\frac{XC}{r} + \frac{AX}{R}$.

If the whole distance from A to D be put $= b$, then $AX = b - x$.

Substituting, we have $\frac{\sqrt{x^2 + h^2}}{r} + \frac{b - x}{R}$,

multiplying by Rr gives us the value

$$S = R\sqrt{x_2 + h_2} + (b - x)r,$$

where $S =$ loss due to friction.

Differentiating and equating to zero we obtain a value for x which makes S a minimum.

Thus
$$\frac{dS}{dx} = \frac{2Rx}{2\sqrt{x^2 + h^2}} - r = 0;$$

$$\frac{r}{R} = \frac{x}{\sqrt{x^2 + h^2}} = \frac{XD}{XC} = \cos\theta.$$

That is, the angle of origin required is such that its cosine is numerically equal to the radius of the branch divided by the radius of the main trunk.

The size of the angle of origin is governed neither by the radius

of the branch vessel nor by the radius of the main vessel, but by the ratio of these two quantities. For any particular value of the ratio r/R, we have therefore a constant value of θ; that is, all branches of equal radius will be equally inclined to the main artery.

(1) In particular, if the artery bifurcates into two equal branches, the angles of bifurcation will be equal.

(2) If r is so small compared with R that the amount of blood going to the branch is almost negligible, then $\cos\theta = \frac{r}{R}$ tends to be infinitely small, *i.e.* angle θ will be close to 90°.

(3) If r differs but slightly from R it is obvious that $\cos\theta$ tends towards the limiting value $= 1$, *i.e.* θ will be very small.

While these statements are true as they stand they are not the whole truth. Other factors come to bear on the angle of origin and produce modifications not comprehended in Hess's Law.

Further Reading

Lovatt Evans. "Recent Advances in Physiology," J. & A. Churchill.

Bainbridge. "The Physiology of Muscular Exercise," Longmans, Green & Co.

CHAPTER XXVI

THE ELECTROCARDIOGRAM

"Providence . . . can make a harmony
In things that are most strange to human reason."
MIDDLETON.

THE electrical changes that occur during each cardiac cycle have, of late, become rather important to the clinician, as a rapid and reliable indication of the state of the heart. Cardiac muscle, just like any other muscle, or, in fact, like any other living tissue, is the seat of electrical differences in potential. Ordinary skeletal muscle on contracting develops potential in such a way that the contracting part becomes electro-positive or zincative to the rest. This causes a current to pass through the external or galvanometric circuit to the contracting part, from the rest of the muscle. Heart muscle acts in a similar way. It has been found that the wave of contraction starts at the sino-atrial node. Therefore, the node will become electro-positive (galvanometrically negative) to the rest of the heart. The atria next contract as a whole, passing on the excitation through a piece of primitive tissue (Bundle of His) to the ventricles. Node, atria and ventricles, as they contract, become electro-positive (zincative) to all other parts of the heart.

(1) **Rheoscopic Frog.** The existence of this change in the sign of the potential developed as the wave of contraction passes over the various parts of the heart may be demonstrated, as it was in muscle (p. 179), by the use of a fresh nerve-muscle preparation. The nerve laid across the beating ventricle produces two muscle twitches per beat.

(2) **Capillary Electrometer.** Earlier experimenters used the capillary electrometer (Fig. 12) as the instrument wherewith to detect *and measure* these potential differences. They found, on leading two electrodes from different points of the atrium, that the amplitude of movement of the mercury produced at each heart-beat is greatest when the line forming the shortest distance between the electrodes would pass through the sino-atrial node. This is interpreted as an indication that the electrical disturbance has its origin at the node.

Consider, for a moment, a large circular sheet of muscle, and near the centre of the sheet are placed two electrodes (*A* and *B*)

leading to an electrometer. If the edge of the sheet is now stimulated at various points, it will be obvious that the greatest movement of the mercury will be produced when the point of stimulation of the muscle lies on the extension of the straight line joining the electrodes. That is, the wave of negativity takes a longer time to pass to *B* when it starts radially opposite to *A* than when it radiates from any other point on the periphery of the sheet. Further, if *A* is placed so near to the point of stimulation that it is practically on it, then no matter where *B* is put, *A* will always be zincative (galvanometrically negative) to *B*.

Evidence as to the origin of the cardiac contraction at the sino-atrial node may be deduced in a similar way from electrometer readings. If one lead is taken from an electrode placed on the

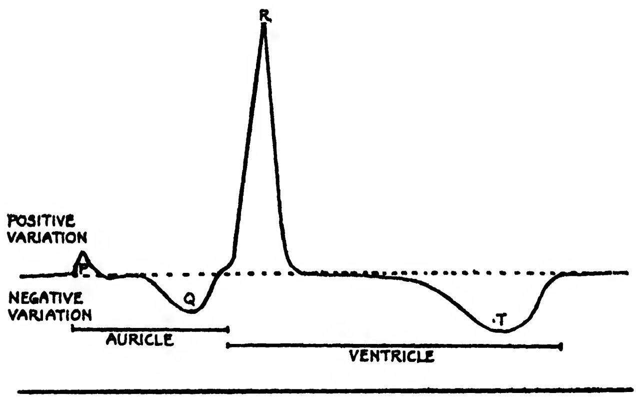


Fig. 90.—Record of the electrical variations in the beating heart of a tortoise, taken by a capillary electrometer (after Gotch).

node and the other lead from an electrode moved about from place to place on the atria, the electrode on the node will be found always electro-positive (zincative).

The figure (90) was obtained by leading one electrode from a point near the atrial sinus and the other from a point near the apex of the ventricle. In order to standardise such records, the leads are always arranged so that any upward movement of the shadow of the mercury (or of the string of the galvanometer) above the line of equal potential (rest) indicates *negativity* (zincativeness). This may be done by leading the atrial electrode to the mercury in the capillary of the electrometer (Fig. 48) and the ventricular electrode to the mercury in the cup. On the initiation of contraction the mercury runs up the capillary away from the tip, indicating that the atrium was electro-positive to the ventricle. This is followed immediately by a tiny downward movement of the mercury, showing that the wave of negativity had passed the site

of the atrial electrode. This constitutes the second part of the atrial diphasic response. Similarly a large upward excursion of the mercury followed by a smaller downward movement demonstrated a similar but greater ventricular diphasic response.

It is not necessary to expose the heart and lay non-polarisable electrodes on it in order to see this diphasic response by the electrometer. The right arm may be considered as electrically continuous with the base and the left leg (or arm) with the apex of the heart.

Using these leads, one may easily identify on the record the *P* wave (Fig. 90) which may readily be shown to correspond to the

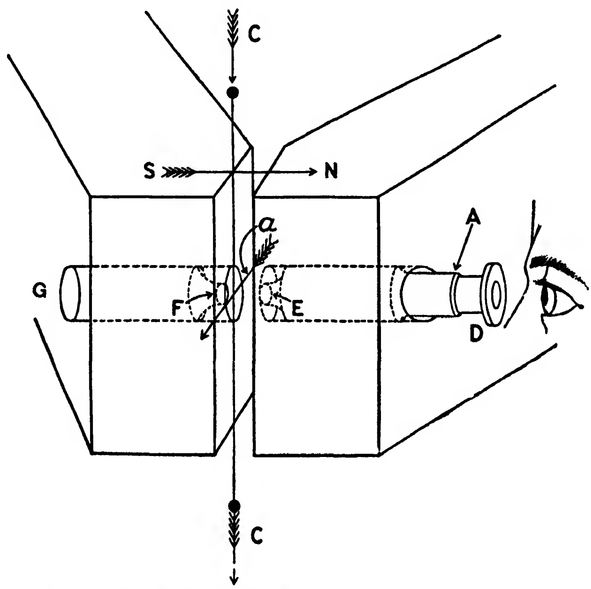


FIG. 91.—Diagram of the essential parts of the string galvanometer. *N* and *S* are the poles of a powerful electromagnet, between which is stretched the fibre *C*.

contraction of the atrium. As potential differences radiate from the heart to the surface of the body, and may even be communicated to the air and detected at a reasonable distance from the body (Potter), leads might be taken from any two points on or near the surface of the body. Certain leads, however, give better results than others, due to the fact that the heart lies obliquely, and, therefore, produces an asymmetrical distribution of lines of equal potential. For this reason and for convenience three pairs of leads have been adopted for standard practice, namely :—

Lead I.—Right arm and left arm.
Lead II.—Right arm and left leg.
Lead III.—Left leg and left arm.

Records from these three pairs of leads differ from one another, and information may be gained from these differences as to the state of the myocardium at various parts.

(3) **String Galvanometer.** Clinicians seem to prefer the more sensitive string galvanometer as an instrument for electrocardiographic work, in spite of its great expense and the difficulty of analysing its records. The instrument at present generally employed is substantially that invented by Ader and modified by Einthoven. The earlier forms of string galvanometer were almost useless as a means of registering the rapid alterations in the electrical state of the heart. Any recording apparatus for such work must be as " dead beat " as possible—moving in exact accordance with the exact potential difference developed and having no period of vibration of its own. As its name implies, the moving part of the string galvanometer is a string or fibre. The string (*C*, Fig. 91), which is an extremely light fibre of silvered glass, quartz, or platinum, is stretched between the poles (*N*, *S*) of a powerful electromagnet. When a current passes along a fibre, the fibre is deflected at right angles to the magnetic field, the amplitude of the excursion depending on the magnitude of the potential difference causing the current; and the direction of the deflection (observer's left or right) depending on the direction in which the current is passing. If

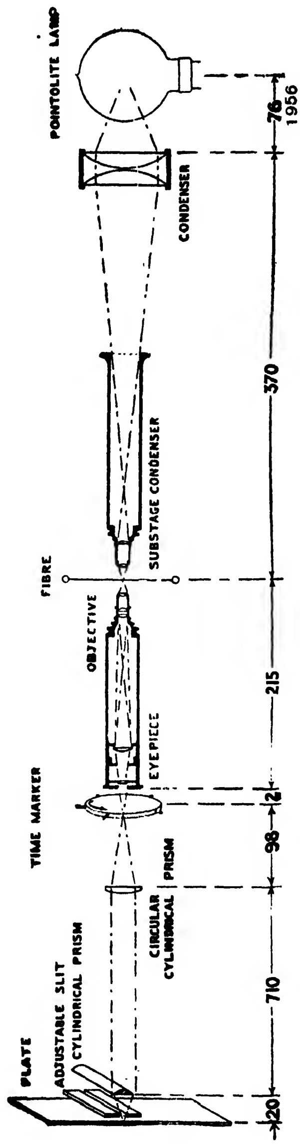


FIG. 92.—Diagram of the arrangement of galvanometer and accessories employed for photographing the fibre movements. The distances are given in millimetres.

the current passes in the direction of the arrow, from top to bottom of the diagram, the fibre will bend outwards, *i.e.* in the direction of the arrow **a**. Reversal of the direction of the current, of course, causes reversal of the movement of the fibre. The excursions of the string can be observed by means of the reading

microscope *AE*, which passes through a hole in the magnet, or records may be made by placing an arc lamp at *G*, concentrating the light on the fibre by a lens *F* and throwing the shadow on to a moving photo-sensitive surface. Fig. 92 shows diagrammatically the arrangements of galvanometer and accessories for photographing the fibre movements. The distances are given in millimetres.

The optical mechanism for producing the electrocardiograms needs some mention. The camera is a light-tight box fitted with a cylindrical lens and an arrangement whereby a sensitive photographic plate or film (or bromide paper) is made to travel at a uniform speed past the narrow lens. The field of the objective

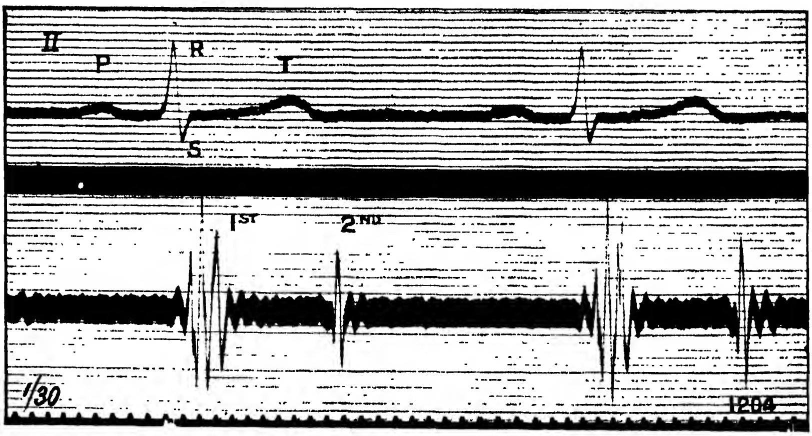


FIG. 93.—Electrocardiogram from lead II. and Phono-cardiogram taken simultaneously from a normal subject.

is projected by an eyepiece on to the lens, which focuses it as a spot of light on the part of the sensitive surface exposed by the slit. The shadow of the fibre appears as a dark spot in this band of light. Thus if the plate or paper be moved downwards normal to the cylindrical lens, the whole surface will be exposed to the action of the light except that portion protected by the shadow of the fibre. The movements of the fibre are, as we have seen, parallel to the plane in which the lens is set, and therefore when the fibre moves towards the reader (in the diagram) the result will be a corresponding alteration in the position of the shadow spot. A continuous record of these positions is formed on the moving sensitised surface.

The record (Fig. 93) shows vertical and horizontal markings as well as the electrocardiogram itself. The horizontal markings enable one to find by inspection the potential difference

generated at different phases of the cardiac cycle. The space between each line is generally 1 mm. = 1/10,000 volt (Einthoven's standard). The lines are engraved across the *width* of the cylindrical lens. When illuminated they produce shadows forming lines along the length of the record. The vertical lines, shortened to ticks at the foot of the record illustrated, are a measure of time—in the case given = one-thirtieth of a second.

They are produced by the interruption of the focused beam of light by a serrated wheel (Fig. 92) so that for a short interval no light falls on the whole (or on part of the sensitised surface) as it is travelling past the slit. In consequence, a sharp line falls on the record.

Before a record can be taken, it is necessary to know the resistance of the subject's body and the magnitude of the "skin-current." The latter factor is a relatively large and fairly constant potential difference caused by the glandular activities of the skin. It has to be counterbalanced by sending an equal current through the fibre in the opposite direction. The resistance of the body to the passage of a current is very rarely considered in routine clinical electrocardiography.

The analysis of electrocardiograms (Fig. 93) is by no means simple. Considerable uncertainty exists as to the exact interpretation of certain units in the trace. If Einthoven's symbols PQRST are used it is generally agreed that P is pre-systolic and that Q (positive E.M.F.) indicates that the wave of contraction does not start at the base of the ventricle but a short distance from it. R is no doubt the wave of negativity produced by the contraction of the ventricles. The upstroke of R is inscribed just *before* ventricular systole starts. S is the second phase or positive reaction of the ventricles. The space between S and T represents the time during which the whole ventricle is excited, and T probably indicates the arrival of the wave of negativity at the apex, culminating at the moment that the ventricles begin to relax. The QRS complex is a composite picture consisting of the algebraic sum of the electrical effects in both ventricles. Other interpretations have been given.

It has been suggested for ease in analysis, that it is advisable to compound the records from all three leads into one diagram. This so-called monocardiogram represents the algebraic sum of all the potential differences at every point of the cardiac cycle.

Consider again the sheet of muscle mentioned above. The line joining *AB* and projected to the periphery will be the *electrical axis* when the point of stimulation lies on it, *i.e.* the electrical axis is the resultant direction of the electromotive changes. It is obvious

that, in the heart, it is not constant, but varies in direction with every phase of the cardiac cycle.

The three leads are represented on paper by the three sides of an equilateral triangle, vertex pointing downwards, and a drawing of the heart is placed in the triangle, having its base on one side (corresponding to lead I.) and its right side (on the left of the drawing) = lead II. Now, if a line be drawn on the heart in the triangle to represent the electrical axis at any moment, and the line be projected by drawing perpendiculars on the three sides of the triangle, then the algebraic sum of the projections on any two sides is equal to the projection on to the remaining side. The projection of the electrical axis will be greatest on that side of the triangle which is more nearly parallel to it. From this mathematical truth Einthoven has formulated the rule that the potential values represented in the cardiogram from lead II. are equal to the sum of the corresponding values obtained in the graphs from leads I. and III. That is, the height of R_{II} (R in cardiogram from lead II.) is equal to the sum of the heights of R_{I} and R_{III}. Knowing the potential values of a given wave, P, R, S or T in the three leads, *the direction of the electrical axis during the production of that wave* can be calculated by use of a trigonometrical formula.

Since the potential values in the three leads are the projections of the line of the electrical axis on the three sides of the triangle, it follows that the maximum manifest value for any wave will appear on the cardiogram when the representation of the electrical axis is parallel to the line representing the lead. It will have a minimum value when the axis line is at right angles to the line of the lead. The magnitude of any wave from any lead, therefore, depends on the angle which the electrical axis makes with the side of the triangle representative of the particular lead taken at that time. That is, the deflection of the string will be greatest in lead I. when the electrical axis is parallel to the base of the heart, in lead II. when parallel to a line drawn from apex to right side of base and making an angle of 60 degrees with it, and in lead III. with a corresponding line on the left side of the heart.

Effect of H ion Concentration. It is well known that the rate at which the excitation is conducted over the heart muscle varies considerably with the *p*H of the fluid medium in a perfused heart. Increase in alkalinity, for instance, increases the conduction rate and decreases the refractory period. Acid, naturally, has the opposite effect. It has been found that the current of injury of skeletal muscle (*q.v.*) can be reversed by decreasing the *p*H below 7·4, the critical level. It is, therefore, suggestive to find that the P, R and T waves of the electrocardiogram can be reversed in sign

by changing to a more alkaline perfusing fluid. This gives us ground for a plausible explanation of the effect of the vagus and of the sympathetic nerves on the rate of conduction in the heart. Some evidence has been produced to show that acid ions are liberated in heart muscle when the peripheral end of the cut vagus is stimulated. The inhibitory power of the vagus is increased also on the addition to the perfusion fluid of certain salts of calcium which are known to dissociate with the liberation of acid ions (see Blood-clotting).

Further Reading

Lewis. "The Mechanism and Graphic Registration of the Heart Beat." Shaw & Son.

CHAPTER XXVII

EXTERNAL RESPIRATION

"The body is sustained by three kinds of nutriment, food, drink, air (πνεύματα), of which the last is by far the most important."

HIPPOCRATES.

FEW of the mechanical arrangements of the body lend themselves better to popular descriptive writing than the lungs, and fewer still have given rise to more misconception of the actual means employed in the performance of their function. From the earliest times of which written records exist, one of the most important and yet most mysterious problems of physiology has been the part played by the lungs. The regular inhalation of air and its regular exhalation was recognised by all as essential to life. Prolonged stoppage of either caused death, and death was accompanied by cessation of breathing. Hippocrates, following Hindu philosophers, maintained that "aerial nutriment" was "the chief support of animal life" (Cicero). Aristotle denied this and considered that the function of respiration was to cool the heart. The followers of Hippocrates, noticing that the arteries and veins differed in structure, suggested that they might differ also in function. It was further observed that the arteries of a dead man were empty although the veins were full. Hence they argued that the arteries were channels for air and not for blood (Erasistratus, *circa* 294 B.C.). That these philosophers had a glimmering of the truth may be adduced from Galen's writings, *e.g.* "The air which is drawn outwards from the rough arteries (trachea and bronchial tubes) receives its first elaboration in the flesh of the lungs, but afterwards in the heart and arteries." It is our business at present to consider the first step in this sequence, viz., the passage of the respiratory gases between lungs and atmosphere.

Principle of Mechanism.

The lung mechanism may be considered as an elastic bag with one opening, the whole suspended in an air-tight box with movable sides. When the sides are pulled outwards the box increases in capacity and the air is sucked into the bag to keep the pressure constant. When, however, the force which drew the sides out-

wards is released, the box and bag resume their former volumes, and air is expelled. In short the lungs are a form of suction pump or bellows (Fig. 94).

Structure of Mechanism.

While the foregoing account of the principle underlying the respiratory mechanism may be taken as substantially correct, it is apt to convey a wrong impression of the details of the mechanism.

(*a*) The lungs are not simple elastic bags, but are composed of thousands of little distensible air sacs—forming an elastic sponge-work.

(*b*) Each lung is subdivided into lobes, the left lung having two lobes and the right lung three lobes, and is in communication with the external air through the *trachea* and *bronchi*. The windpipe, or *trachea*, divides into two bronchi, one of which, with the pulmonary blood and lymph vessels, etc., enters each lung at the *root* of the lung. The *bronchi* on entering the lungs undergo repeated branchings, and finally each tiny *terminal bronchiole* subdivides into a number of *alveolar ducts*. On these are little expansions, the *atria*, from which the air sacs or *alveoli* open. Each bronchial branch is accompanied by a branch of the *pulmonary artery*, which ultimately breaks up into a fine network of blood capillaries on the walls of the *air sacs*. The blood from the capillaries is returned to the left side of the heart by the *pulmonary veins*.

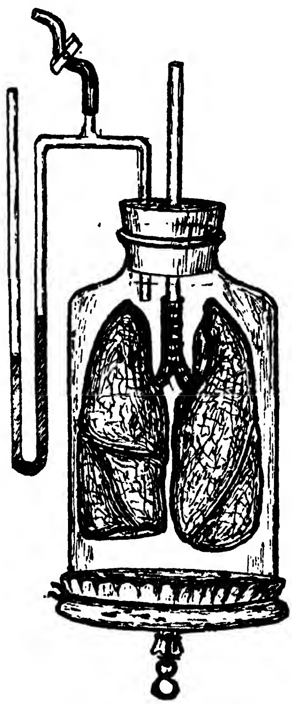


FIG. 94.—Model to demonstrate action of diaphragm. On pulling the rubber sheet downwards, air enters the lungs and they expand.

(*c*) These complex bags are suspended in and almost fill the thoracic cavity. Each lung is enclosed in a membranous sac—*the pleura*, which, on reaching the root of the lung, bends back from the bronchi and lines the entire internal surface of the chest wall. Each lung has, in its development, pushed into a closed sac, and, carrying the walls of that sac with it, has been completely enveloped by it. That is, the pleura consists of two layers—an outer, parietal or chest wall layer, and an inner, visceral or lung layer. The surfaces of the two layers are kept moist with lymph. *It is important to note that as long as the chest wall is kept intact the pleural cavity is only a cavity in name.* The layers of the

pleura are always normally in close contact with one another and with the underlying and overlying surfaces. In other words, the chest wall, the two layers of the pleura and the outer surface of the lungs move almost as one structure. The elasticity of the lungs has been determined as about 30 mm. Hg. If this inwards pull of the pulmonary tissue be subtracted from the atmospheric pressure (760 mm.) in the lung, the resulting figure (730 mm.) represents the force tending to keep the lungs expanded. If, now, communication be established between the outer air and the intra-pleural cavity, there will be a pressure of 760 mm. tending to cause the lungs to collapse. As these outwards and inwards pressures (760 as against 730 mm.) do not balance, one would expect to find that the lungs collapse. This is not always so. A further force comes into play. Moistening the various surfaces is the lymphatic secretion already referred to and, by the force of surface-tension, the lungs are held to the chest wall, just as firmly as a boy's leather " sucker " is held to the pavement and for the same reason.

Mechanics of Respiration.

During inspiration the capacity of the thorax is increased in all directions. That expansion occurs laterally and in an antero-posterior direction may be made manifest by measurement or by moulding strips of lead (cyrtometers) to the circumference of the chest. The movements in a vertical plane have been studied by means of the X-rays and by percussion. If the intercostal spaces are tapped with the finger, a clear resonant note will be emitted when the percussion has been performed on a part overlying inflated lung. Otherwise a dull sound will be produced. Horizontal expansion is obtained by movements of the ribs while the vertical movements are caused by contraction of the diaphragm.

I. **Structure of the diaphragm.** This is a vaulted musculo-fibrous sheet separating the thorax from the abdomen. It consists of a central tendon like a double-arched cupola which is attached on its thoracic surface to the pericardium and marginally to the thoracic walls by muscles. These diaphragmatic muscles may be divided into two sets, (i.) *crural* and (ii.) *costal.* The former have their origin in the three or four lumbar vertebrae and in the arcuate ligaments and are inserted into the posterior margin of the central tendon, while the latter arise from the cartilages and lower six ribs and from the back of the ensiform process. Such a division of the muscle into crural and sterno-costal portions is supported not only (1) by their different *origins*, but (2) by their *development* from different muscular sheets in the embryo ;

(3) by their different *blood supply*—the former directly from the aorta and the latter from the intercostal and internal mammary arteries; and (4) by their different *nerve supply*, the crural being served by the posterior branch of the phrenic nerve and the costal by the anterior branch. Moreover, the two portions act somewhat differently, and further, people may be classed as having respiration of a crural or of a parietal type depending on whether the crural or the costal portions of the diaphragm are employed during quiet breathing. The majority of individuals employ both parts of the muscle in varying degrees.

II. Mechanics of diaphragm. The crural portion, when it contracts, acts as power to a lever of the third class. That is, the fixed point or fulcrum is the point of origin of the sheet of muscle on the vertebral column. The resistance to be overcome is mainly the pressure of the contents of the abdomen, the pericardial fixture and the point of insertion of the *vena cava* and other vessels. They may, on the whole, be considered as a weight applied at the central tendon. The power is thus between weight and fulcrum—giving speed at the expense of strength. The sterno-costal part of the muscle connects the lower ribs with the central dome and acts as a lever of the same class as the crura. In this case, however, the fulcrum is movable and is moved outwards by other muscles. This results in a forward as well as a downward movement of the dome.

On the whole, the final result of the contraction of the diaphragm is similar to the descent of a piston—increasing the capacity of the thorax vertically. The average descent is equivalent to a drop of about ½ in. all over. For ease in calculation, say that the distance through which the diaphragm moved in an ordinary quiet respiration were 10 mm. and that the mean area of the piston were 250 sq. cm., then the volume of air sucked in would be 250 c.c. (complemental pleura). Now as the tidal air in quiet breathing is under 400 c.c., it will be clear that the part played by the diaphragm in ordinary respiration is of major importance.

Synergic Muscles. Acting along with the diaphragm there are those muscles which abduct the lower ribs, viz.: the *quadratus lumborum* and the deep costal muscles. These are synergic—contracting synchronously with the diaphragm, and preventing the lower ribs from being pulled inwards. In children where the musculature is poorly developed one sometimes observes a distinct depression of the lower chest wall at every inspiration.

The **antagonistic muscles** together with the viscera form the resistance against which the diaphragm moves. These are the

muscles of the abdominal wall, viz.: external oblique, internal oblique, transversalis and rectus abdominis on each side.

The floating ribs (and in 40 per cent. of people, the tenth rib also) are functionally part of the abdominal wall. Their movements are controlled by the *quadratus lumborum* and *erector spinae* muscles. The twelfth rib, in addition, is anchored to the transverse processes of the first and second lumbar vertebrae by a strong ligamentous membrane, an extension of the middle layer of the lumbar fascia. In this way, the upward movement of the rib, especially in its spiral segment, is restricted. The anterior and lateral segments have a freer movement, so permitting of a movement of the floating ribs (and the tenth) round an axis corresponding to their spinal segments. It has been noticed that during inspiration the spaces between those ribs widen and that during expiration the reverse takes place.

Function of Abdominal Muscles. The four pairs of abdominal muscles and their fibrous attachments act antagonistically to the diaphragm. When the latter contracts, the former have to yield to accommodate the displaced viscera. That is, during diaphragmatic breathing, inspiration is accompanied *by a relaxation of the abdominal wall which will move forwards.* Correspondingly, expiration will be aided by the tendency of the viscera to return to their normal positions and by the return of the abdominal muscles to the position of rest.

This musculature has also an important part to play in the maintenance of an adequate circulation. There is no doubt that the diaphragm, with its synergic and antagonistic muscles, was evolved not in connection with respiration, but with circulation. Amphibians, for instance, carry on their interchanges of air between lungs and atmosphere by the action of muscles under the jaw. In the mammal, without the constant tension of the abdominal muscles applied to the abdominal viscera, the larger veins would become distended with blood, and these veins are capable of holding the entire amount of blood in the body. So, if for any reason the muscles of the abdominal wall lose tone, a considerable fall in arterial blood pressure is the result. It may even fall to zero and death ensue. This may be determined experimentally, either by dividing the spinal cord at the level of the first thoracic vertebra, or by using an animal with poorly developed abdominal muscles such as the tame rabbit. In the first case, the influence of the bulbar centres on the part below the section is removed, and the tone of the abdominal wall is abolished. If the animal is now placed vertically erect, the abdominal veins distend under the haemostatic pressure. In them such a large

proportion of the blood collects that there is insufficient blood to fill the heart.

III. Thoracic Respiration. The upper and lower regions of the thorax should be considered separately. The muscles and movements of the upper series of ribs are quite different from the lower series.

(*a*) Lower costal series (sixth to ninth or tenth rib).

This segment moves along with the diaphragm and leads to the expansion downwards of the lower lobes of the lungs. The ribs are articulated to the spinal column so that during inspiration the lateral and anterior part of each moves outward more than the one above it. Two movements may be noted :

(i.) The 50 to 70 mm. of each rib next the spine to which is attached the *erector spinae* muscle moves forward at each respiration. The tubercle of the rib slides forward on the flat upper facet of the transverse process.

(ii.) The non-spinal portion of a pair of ribs moves with a bucket-handle action, rising and coming forward with each inspiration. At the centre of each pair is the sternum-cartilage complex which is raised and forced forwards during inspiration. The muscles concerned in this increase of the volume of the lower thorax, transversely and antero-posteriorly, are the external intercostals.

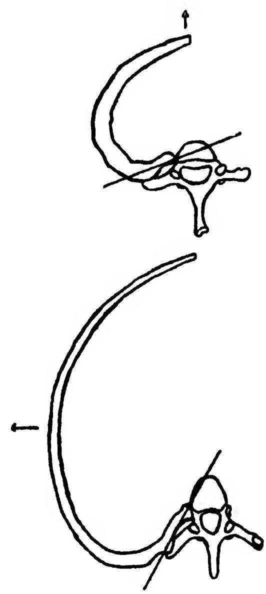


FIG. 95.—Rib and vertebra in upper and in lower costal series to show the difference in the obliquity of articulation and the resulting difference in the expansion of the chest. Note direction of arrows. (From Noël Paton's "Essentials of Human Physiology.")

(*b*) Upper costal series (second to fifth rib).

These ribs differ from the lower series in shape, articulation, ligamentation, musculature and, consequently, in their movements.

(i.) *Shape.* The upper ribs have a concave upper margin and do not have such a marked twist as those in the lower costal series. The second rib as a matter of fact may be laid flat on a table.

(ii.) *Articulation.* The spinal articulation differs from the lower series mainly in that the convex ovoid facet of the tubercle fits into a corresponding cavity in the transverse process instead of gliding on a flat facet. The costal articulations are nearly in a transverse axis (Fig. 95), and movement occurs at the manubrio-

sternal articulation. Each transverse process from above downwards is tilted a little more backwards so that the angle of articulation becomes more oblique as one passes down the series.

(iii.) *Ligamentation.* Each of the upper series of ribs is joined directly to the sternum by a band of cartilage. The following are the lengths of these attachments in a well-built man: second, 37 mm.; third, 50 mm.; fourth, 62 mm.; fifth, 75 mm. The angle of attachment increases as the length increases, *e.g.* the second costal cartilage joins the sternum at right angles while the third ascends to the sternum.

(iv.) *Musculature.* The musculature of these ribs is the intercostal interchondral and external intercostal.

(v.) *Movements.* Because of the nature of the articulation of each rib to the vertebral column by tubercle and head, rotation round a spino-sternal axis is limited. *Very little bucket-handle action can take place.* As the articulations are practically transverse, movement must occur at the manubrio-sternal articulation, *i.e.* chiefly forwards.

(*c*) **The first rib** provides the necessary fulcrum for the intercostal muscles. Along with the manubrium sterni, to which they are firmly bound by their broad but short costal cartilages, the first pair of ribs form the *operculum* or lid of the thorax. This lid is articulated anteriorly with the thoracic wall, at the manubrio-sternal joint, forming a *synchondrosis.* That is, the opposing surfaces of bone covered with a layer of hyaline cartilage and united by fibro-cartilage are bound together firmly by longitudinal fibres developed from the strong and thick periosteum. The limitation of movement thus imposed at the joint is counterbalanced by the greater freedom of movement which is allowed at the articulation of the heads of the first pair of ribs with the thoracic vertebra.

Great importance has been attached to the movements of this joint. Its amplitude varies, of course, with the type of respiration, being greatest with those who make least use of the muscles of the abdominal wall and *vice versâ.* In other words, if the sternum moves freely then the excursions of the sterno-manubrial joint will be small. On the other hand, in cases where the lower part of the sternum moves but little during inspiration (thoracic breathing), there will be a correspondingly large rotation of the upper end of the sternum on the end of the manubrium. Some physicians declare that in phthisical subjects this joint does not move freely. Whether phthisis causes an anchylosis or whether want of free movement leading to incomplete expansion of the

apices of the lungs is a factor favouring the development of the disease, is as yet an unsolved problem. On the whole the evidence tends to show that ossification of the costal cartilages in question is a consequence rather than a cause of a limited expansion of the apices of the lungs.

Posteriorly the lid is articulated to the vertebral column by a joint which is set more transversely and is wider in the extent of its attachment than any other of the costal arcs.

IV. **Mechanics of Thorax.** The ribs are a series of bent levers.

(1) The **fulcra** or hinges on which the levers work have been mentioned when dealing with the ribs of the various thoracic segments.

(2) The **power applied** differs according to whether inspiration or expiration is being performed (p. 423).

(*a*) Inspiration.

(i.) The lid or operculum is raised by the action of a flat triangular muscle (*scaleni*). The *scalenus anticus* is inserted into the inner border of the first rib and passes almost vertically to the transverse processes of the third, fourth, fifth and sixth cervical vertebrae. The *scalenus medius* lies posteriorly to the anticus and passes to the transverse processes of the lower six cervical vertebrae.

(ii.) The external intercostal muscles may be regarded as a triangular sheet of muscle having its origin in the posterior part of the lid and being inserted into the upper surfaces of the ribs. It pulls upwards.

(*b*) Expiration.

(i.) The power causing collapse of the chest wall is mainly the elastic recoil of the lungs together with the weight and elasticity of the chest wall.

(ii.) The abdominal muscles, especially the *external oblique*, play a part in expiration in pulling down the ribs. The fixed basis from which they act is the pelvis, and they act as if attached to the lower margin of the ribs exactly opposite the external intercostals.

(3) **Load.** This, too, is different in inspiration and expiration.

(*a*) Inspiration.

The resistance to be overcome is :—

(i.) The elasticity of the lungs—a variable load, as the greater the expansion of an elastic body, the greater is the resistance that it offers to further expansion. This factor, therefore, is numerically greater towards the end of inspiration than at the beginning.

(ii.) The elasticity of the chest wall—the costal cartilages have

to be twisted and the muscles overlying the chest wall have to be stretched.

(iii.) The elasticity of the abdominal wall.

(iv.) The elasticity of the vertebral column. During inspiration the spinal column is lengthened by a stretching of the ligaments, cartilages and articular processes.

(v.) Gravity—weight of chest wall, etc.

These loads may be resolved into one applied to the upper surface of the ribs at their frontal tips. That is, we are dealing with levers of the *third* order where power is applied between load and fulcrum—giving speed at the expense of strength (Chap. XXX.).

(*b*) Expiration.

The main resistance to expiration is the resistance to the outflow of air from the lungs. We have seen that the principal force causing expiration is the inspiratory load. Here then we have a lever of the *second* class with the load between the power and the fulcrum. During forced expiration, when every muscle that can reduce the size of the thorax is brought into play, we have a simple bellows action. The front of the thorax acts like the movable side of a pair of bellows and is depressed towards the other side by the abdominal muscles. This is also a lever action of the *second* order.

V. Elasticity of the lungs. The work done by the respiratory musculature cannot be treated as a simple problem in hydraulics. The dynamics of the ordinary force pump cannot be applied to this question. Not only are the walls of the pump elastic and complex, but (*a*) they are not equally extensible throughout and (*b*) their elastic force varies with the degree of extension. Further, (*c*) the fluid enmeshed in the pulmonary capillaries has to change its position to be accommodated at every alteration in the extension of the lungs.

(*a*) Examination of the structure of the lungs shows that they cannot be equally extensile throughout. Anatomists divide each lung into three zones.

(1) Root zone containing bronchus, artery, vein, lymphatic vessels, etc. This part contains much fibrous tissue and, therefore, offers considerable resistance to distortion. Using physical terms one may say that its elasticity is *strong*, but far from *perfect* (p. 206).

(2) Outer zone, estimated as extending for about 30 mm. from the pleura containing very little fibrous tissue and made up mostly of small capillaries and pulmonary tissue. Of these the pulmonary tissue is *perfectly* but *feebly* elastic and the capillaries (empty) have a modulus of about $0{\cdot}04 \times 10^6$—not quite so perfect as the lung

substance, but offering a greater resistance to distortion. Even within this zone extensibility is not uniform. The stratum lying immediately below the pleura is much more extensible than the inner stratum. Inflation of a lung recently removed from the body clearly demonstrates that certain parts of the surface are inflated first and that the inflation of certain parts of the sub-pleural stratum spreads from these points.

(3) The middle zone, lying between the root and surface zones, is intermediate to them in its elastic properties, containing as it does highly elastic pulmonary tissue interspersed between the rays of the bronchial and vascular systems.

(*b*) That the elastic force of a material alters with the degree of distension is a physical fact that has already been considered in dealing with the force of the heart. Since the pressure of a gas acts equally in all directions, the pressure caused by any given tension of the walls of the hollow (spherical) vessel containing air will increase with the diameter of the vessel. If we consider that the diameter of each air sac is doubled during inspiration, then the total pressure exerted by the walls will be increased four times, *i.e.* distending force = resistance to distension = pressure of gas multiplied by area of vessel. Moreover, with increasing distension, the lung substance will become more attenuated.

(*c*) The blood and lymph enmeshed in the pulmonary system has to adjust its position to suit every alteration in the shape of the lungs. These fluids are highly viscous, and as such resist distortion roughly in proportion to their pressure and to the area of the cross-section of their vessels. Further, the capillary vessels are so narrow that the corpuscular component of the blood viscosity becomes predominant.

(*d*) In addition to these factors which may be deduced from a study of lungs removed from the thorax one must take into consideration the position of the lungs in the thorax. Certain parts of the thoracic wall are stationary, and the surfaces of the lungs in contact with these parts cannot *directly* expand, viz.—

(i.) the *mediastinal surface* in contact with the pericardium and with the structures of the mediastinum. (ii.) the *medial surface* lying close against the vertebral column and spinal portions of the ribs and its *anterior portion*, in contact with the mediastinal pleura. (iii.) *The posterior part of the apical surface* is bounded by Sibson's fascia at the root of the neck.

On the other hand the parts of the lungs in contact with (iv.) the diaphragm, (v.) the lower ribs (ventro-lateral aspect) and (vi.) upper ribs (sternal aspect) undergo *direct* expansion at each inspiration.

VI. The efficiency of the lung mechanism. If figures could be

obtained denoting the work done by the respiratory mechanism and its efficiency, they would be invaluable. One may arrive at an approximate value by measuring the oxygen consumed by an animal under standard conditions with normal and with increased respiration. With man, it was found that during muscular rest, 1 to 3 per cent. of the total basal oxygen intake is utilised by the respiratory mechanism. This amounts to from 0·3 to 0·9 c.c. of oxygen per litre of ventilation. Assuming that all the energy used is obtained from glucose, these figures indicate that from 0·0015 to 0·005 Calorie is expended for each litre of air breathed. This amount of energy is liberated from 0·0004—0·0012 gram of glucose. During quiet breathing each breath (400 c.c.) costs at most 0·002 Calorie obtained from just about 0·0005 of a gram of glucose and 0·36 of a cubic centimetre of oxygen. If it is assumed that the lung mechanism is at least 20 per cent. efficient, then at each quiet complete respiration, 0·00014—0·0004 Calorie is converted into work = 0·06 to 0·17 kilogram-metre.

This work is almost entirely performed by the diaphragm. The other muscles concerned, whether synergic or antagonistic, seem to play an almost passive part. This may be inferred from the fact that although they are skeletal in structure yet they undergo constant slow contraction without showing fatigue. When the respirations are forced the subsidiary musculature has to perform work and the CO_2 output increases. The effort sooner or later brings on fatigue. Forced respirations are carried out uneconomically, *i.e.* at a relatively higher cost per litre than ordinary quiet ventilation (see Voice, Chap. XXVIII.).

Regulation of Respiratory Rate. The activity of the respiratory centre, which lies in the medulla near the root of the vagus, is normally governed by the tension of the CO_2 in the blood going to it. The rate of breathing is increased by any increase in the CO_2 tension; and, conversely, diminution of the CO_2 tension leads to a decreased respiratory rhythm.

The CO_2 tension of the blood and the partial pressure of the CO_2 in the alveolar air, as we have explained (Chap. XXIV.), are always in dynamic equilibrium, and, therefore, any change in the one will lead to corresponding changes in the other. It has been found that an increase of 0·2 per cent. in the CO_2 of the alveolar air of man, *i.e.* a rise of tension of from 40 to 41·6 mm. Hg, is sufficient to double the alveolar ventilation. The increased ventilation leads to a " washing out " of CO_2 from the blood and from the lung, thus rapidly restoring a normal condition. The power of adjustment is so extraordinarily effective that under wide variations of metabolic and atmospheric conditions, the tension

of CO_2 in alveolar air is maintained at an almost constant level of about 40 mm. Hg (see Chap. XXXI.).

Regulation of Depth. Impulses are constantly passing from the lungs, through the vagi, to the respiratory centre and, by a reflex act, inspiration is checked when a certain tension is set up in the lung substance, *i.e.* the respiratory mechanism carries out the inspiratory phase of its function till this stretch-reflex inhibits it. In cases where the vagi are hyper-irritable, the stop mechanism acts too soon and breathing becomes shallow and rapid.

Effect of the Respiratory Movements on Mass Movements of the Blood.

Thoracic Respiration. During each inspiration the increase in the capacity of the thorax not only tends to produce a negative pressure in the trachea and pharynx, so causing air to be sucked into the lungs, but negative pressure is effectively applied to the thin-walled veins in the thoracic cavity, causing them to dilate with blood aspirated from the extra-thoracic venous system. This dilatation causes more blood to collect in the intrathoracic vessels, and to some extent delays the passage of this blood to the atria. During expiration, however, positive pressure is applied to these veins; the extra blood is forced into the heart. As we have seen in a previous chapter, the increased venous filling leads to an increased ventricular output and a rise of blood-pressure.

Diaphragmatic Respiration. During inspiration the diaphragm contracts and presses down on the abdominal viscera. They, in turn, pass on the pressure to the blood in the *vena cava*, with the result that, at first, more blood is forced into the thoracic *vena cava*, and, consequently, to the right atrium; and the blood pressure tends to rise a little. Continued compression of the abdominal *vena cava* cuts down the supply of blood to the thorax, and blood pressure falls. During the expiratory relaxation of the diaphragm the reverse process takes place.

Most people breathe diaphragmatically with a slight rising of the thoracic cage. The effect on their blood pressure will, therefore, be the algebraic sum of the two opposing effects: viz. an initial rise due to thoracic negative pressure and abdominal pressure on the veins. This rise, which would continue in purely chest breathing, is cut short by the fall of pressure due to continued abdominal pressure.

Exercise. The above factors are more marked in their influence on cardiac output during ordinary muscular exercise. There are certain types of exercise, however, in which, in order to produce a maximum leverage (*q.v.*), the chest wall is fixed and respiration is

suspended. For example, in attempting to push a heavy body, pull against an almost unyielding resistance, or lift a heavy weight, a deep inspiration is taken and then, by powerful muscular action, the whole body is knitted into a single lever with a long power arm. The contracted abdominal muscles press the diaphragm, etc., up into the thoracic cavity. This compression at first causes blood to be squeezed from the abdominal veins into the thoracic veins and so into the heart, producing a marked rise in arterial pressure. Later there is a damming back of blood into the peripheral vessels, causing them to stand out like knotted cords. When the effort ceases and the compression on the abdominal contents is released, the large abdominal and thoracic veins fill up with blood, producing a marked fall in arterial pressure. The tissues during the period of sustained effort have removed oxygen in large quantities from the blood in the peripheral vessels, and if the effort is long continued an oxygen debt is incurred (*q.v.*). Marked cyanoses may even develop.

Modifications of the Respiratory Act. A similar positive pressure is brought to bear on the veins entering the thorax during such acts as coughing, sneezing, defæcation and parturition. In the first stage of coughing and in defæcation, after a forced inspiration, the *glottis* (*q.v.*) is closed and the expiratory muscles put into strong contraction. The enormous positive pressure produced, in the former case, in thorax or abdomen, and, in the latter case, in the abdomen, dams back the blood in the veins, causes a fall of arterial pressure, and, if sustained, cyanosis follows. In sneezing, the air is compressed after a forced inspiration by the contraction of the pillars of the fauces, descent of the soft palate and pressure of the tip of the tongue against the hard palate. The effect on blood pressure is similar to that of coughing. Sighing and yawning, which are alleged to be controlled by separate centres in the medulla, are deep inspiratory acts almost entirely thoracic in character and, therefore, tend to encourage diastolic filling and a general increase of blood pressure.

Influence of Heart Action on Respiration.

As the heart contracts and dilates it must alternately decrease and increase the intrapleural pressure, causing a slight inrush and outrush of air with each cycle. These cardio-pneumatic movements may be demonstrated in a very simple manner by filling the mouth with tobacco smoke, inserting a glass tube about 18 in. by $\frac{1}{2}$ in. in the mouth. Hold the tube vertically (preferably with the upper end gently plugged with cotton wool). Allow a little smoke to enter the tube and then hold the breath. The

column of smoke will be seen to pulsate. These movements may be timed with the pulse.

If a simultaneous record be obtained of the heart beat and of the movements of the air in the external respiratory passages (*e.g.* nares), it will be seen that there are three phases of the cardiac cycle in which the influence of the heart on the movements of the air column are manifest :—

(1) At the beginning of ventricular systole intrapleural pressure is suddenly increased. At this moment the ventricles are closed cavities.

(2) During ventricular systole intrapleural pressure decreases as systole proceeds.

(3) During ventricular diastole there is a gradual increase of intrapleural pressure.

These phases may be seen easily in the smoke-filled tube referred to above, viz. (1) smoke-level suddenly rises when the heart beats ; (2) smoke-level drops ; (3) smoke-level slowly rises. During the period of passive diastole the smoke-level remains steady.

If a bronchitic patient has a plug of mucus in a small air passage near the heart, every time air is forced past the plug by the cardio-pneumatic movements the air will be thrown into vibration, and a murmur will be heard very similar in character to a cardiac murmur.

It is alleged that the movement of air produced in this way by heart action is quite sufficient to keep up the necessary gaseous exchange in hibernating animals.

CHAPTER XXVIII

THE VOICE

"As the power of the vital soul is situated in the substance of the heart, and the power of the natural soul in the proper substance of the liver, . . . so also does the brain, in appropriate structures and in organs properly subserving its work, manufacture the animal spirit, which is by far the brightest and most delicate, and indeed is a quality rather than an actual thing. And while on the one hand it employs this spirit for the operations of the chief soul, on the other hand it is continually distributing it to the instruments of the senses and of movement. . . ."

VESALIUS (1543) quoted by FOSTER.

THE production of sound by the larynx or by the lips is so essentially a modification of respiration that we may conveniently deal with phonation, whistling and speech at this point. By means of sound production we act on matter at a distance, and so one would naturally consider the problems of phonation, and especially of articulation, after one had reviewed the various types of levers, pulleys, etc., by means of which the body reacts on its environment.

I. Phonation. At least two essential factors are concerned in phonation, namely, (*a*) a means of producing a flow of air, and (*b*) a means of interrupting that flow so that alternate condensations and rarefactions follow one another in the air at a certain rate. The lungs provide the current of air, and either the sudden expansion of the bore of the wind-pipe at the ventricle, the pursing of the lips to form a small orifice (whistling), the juxtaposition of tongue to palate and closure of teeth (hiss), or the vibrations of the vocal cords account for the breaking of the continuous column of air into waves.

Mechanism of Larynx. The larynx or sound box is situated between the root of the tongue and the trachea. Above, it opens into the laryngeal part of the pharynx, of which it forms the anterior wall; below, it is continuous with the trachea. It is composed of nine cartilages, three single and three paired. They are connected by ligaments and membranes, and moved by some ten muscles—some of which open and close the glottis and some regulate the degree of tension of the vocal ligaments. The larynx is lined by a mucous membrane which is continuous above with that of the mouth and pharynx and below with that of the trachea. Except over the vocal folds, where it is stratified, the epithelium is

of the ciliated variety. Except also on the free edges of the vocal folds the mucous membrane is studded with mucous glands. These are especially plentiful upon the epiglottis (*q.v.*) where they are lodged in little pits.

The cavity of the larynx is divided into three parts by two pairs of folds of the mucous membrane which project from the sides of the cavity into its interior. The upper or ventricular folds are the so-called false vocal cords. Each encloses a narrow band of fibrous tissue (the ventricular ligament), which is fixed in front to the thyreoid cartilage and behind to the arytænoid cartilage. The fissure between the false cords is termed the *rima vestibuli.* The lower or vocal folds are the true vocal cords. Each encloses a band of yellow elastic tissue (the vocal ligament), which is fixed to the same cartilages as the corresponding ventricular ligament, but a little lower down. The vocalis muscle lies lateral to and parallel with the vocal ligament. The fissure between the true cords is called the *rima glottidis.* Between the false and the true cords is a recess known as the ventricle of the larynx (or of Morgagni).

False Cords. The ventricular folds play only a protective part in phonation, keeping the true cords moistened by the secretion of the numerous mucous glands with which they and their appendices are provided. They are of use in " holding the breath." Animals which have to do this often in fight or flight have well-developed ventricular folds.

True Cords. The alterations in the width and shape of the *rima glottidis,* brought about by the movements of the vocal folds and arytænoid cartilages during phonation, can be studied readily by the use of the laryngoscope. This instrument consists of two mirrors, a small one which is held by means of a long handle at the base of the *uvula* with the mirror directed at an angle towards the larynx, and a larger mirror with a central hole through which the observer examines the image in the small mirror. The practical details of this method of examination are given in any practical text-book of physiology (" Practical Physiology," by Anrep and Harris) and are out of place here.

In order that the vocal cords may set the air current into vibration they must be put into a state of tension. In the dead larynx it is possible to produce sounds by forcing air from bellows through the larynx, meanwhile applying tension to the cords by pulling the arytænoid cartilages backwards.

From experiments on the cadaver and on tracheotomised patients it has been found that with a constant tension on the cords sounds vary in loudness and in pitch with the air pressure developed. In the following table (LXI.) is given the limits of

oscillatory pressure (R.M.S.) for various frequencies of vibration detectable by the human ear (*q.v.*). Starling states that in patients on whom tracheotomy had been performed, the pressure of air in the trachea necessary to cause the production of an audible sound was from 140 to 240 mm. H_2O, and for loud shouting a pressure of 945 mm. of water was necessary. This pressure is furnished by the contraction of the expiratory muscles (*q.v.*).

TABLE LXI

NOTATION, FREQUENCY, PRESSURE AND AMPLITUDES OF AIR WAVES OF THE MUSICAL SCALE

Notation.		Frequency.	R.M.S. Oscillatory Pressure.		Amplitude.	
Helmholtz (European).	Sabine (U.S.A.).	Double* Vibrations per Sec.	For Minimum Intensity. Dynes per sq. cm.	For Maximum Intensity. Dynes per sq. cm.	For Minimum Intensity. cm. × 10^{-8}	For Maximum Intensity. cm. × 10^{-2}
C^{I}	—	32	1·0	—	17,000	—
C	C_1	64	0·15	300	1,200	2·5
c	C_2	128	0·03	900	120	4
c^{I}	C_3	256	0·006	3,000	12	6
c^{II}	C_4	512	0·002	6,000	2	6
c^{III}	C_5	1,024	0·0009	4,000	0·5	2
c^{IV}	C_6	2,048	0·0006	1,500	0·2	0·4
c^{V}	C_7	4,096	0·0007	—	0·1	—
—	—	8,192	0·0015	—	0·1	—
—	—	16,384	0·1	—	3·2	—

* In France frequency is measured in single vibrations, and so all the figures in this column are half the French values.

The first muscular act in breathing for the purpose of phonation is a slight inspiration. When this is done properly, the column of air, resting as it were on the diaphragm, is ready for its impact on the vocal cords, an impact which must be made with the greatest nicety and control. Hudson-Makuen states that the expiratory act necessary for phonation is produced by a contraction of the diaphragm which pulls the lower ribs downwards and inwards, *i.e.* a muscle which in ordinary breathing is inspiratory, here acts in the opposite sense. Proper co-ordination of the intercostal and abdominal muscles are, of course, just as essential for phonation as for respiration.

Loudness is due to amplitude of vibration, and depends, in part, on the force and volume of the blast of air emitted.

In part, it depends on the size of the larynx and of the resonating chambers, and on the tension of the vocal cords. A true crescendo is obtained by relaxing the tension of the vocal cords.

Pitch, or tone-height, is a function of the frequency, *i.e.* rate of vibrations. This may be proved by running a gramophone record at various speeds. When running at its slowest the plate of the sound box is receiving and transmitting vibrations at the rate of about 100 per second, and one hears a bass voice. As the speed is increased the voice rises in pitch till it may be a distinctly light tenor with 500 vibrations per second.

The average limits for the human voice are given in Table LXII.

TABLE LXII

(Sabine's Notation)

Bass . .	F_1 to D_3	85 to 288 vibrations per second.
Baritone .	A_2 to F_3	106 to 340 ,, ,, ,,
Tenor . .	C_2 to A_4	128 to 424 ,, ,, ,,
Alto . .	E_2 to C_4	160 to 512 ,, ,, ,,
Soprano .	B_3 to G_4	240 to 768 ,, ,, ,,

We derive the musical notion of *high* and *low* pitch from the rise and fall of the larynx in the production of sounds—an entirely subjective phenomenon. The pitch of the voice may be altered by voluntarily lowering the position of the larynx during phonation—a knack acquired by training. Hudson-Makuen has used this fact in correcting the high-pitched or eunuchoid voice which occasionally occurs in men otherwise normal. A somewhat similar process occurs in the acquirement of the deep voice cultivated by many women who have occasion to lecture regularly. Prolonged use of the lower register gives these people a peculiar " falsetto " booming voice. *The pitch of a tone, from a physical point of view, is absolutely defined by its vibration number.* High-pitched notes have a higher rate of vibration than low-pitched notes. Alterations in the pitch of a note are probably brought about in the larynx by altering the tension of the vocal cords, by the action of the crico-thyreoid muscle—the greater the tension of the vibrating membrane, the higher the pitch of the note produced. The part of the cords free to vibrate may be varied by the approximation of the arytænoid cartilages to one another. A long cord vibrates more slowly than a short one. This accounts for the high-pitched voices of children.

In addition to this, it is common knowledge that when the force of the blast of air is increased, the pitch of the voice rises. There is thus a tendency to sing sharp when forcing the voice, say in a large badly built hall.

In one and the same larynx, different parts or regions of the scale are produced in different ways. Those notes of the scale which are produced by the same means are said to be produced in the same *register*. Thus, we produce deep notes in the chest-,

or thick-register, while high notes come from the high-, head-, or small-register. The thin or middle register is used normally by tenors and when the male voice sings falsetto.

Laryngoscopical investigation has shown that, when producing notes from the chest register, the glottis forms an elongated slit and the vocal cords, stretched as tightly as possible, are vibrating as *thick* masses over their whole extent. In taking the lowest notes the posterior portion of the arytænoid cartilages are close together with a wide elliptical chink between the cords. As the pitch of the note rises the arytænoid cartilages are brought closer together and so shortening of the vibrating portion of the cords is produced. The thyreoid cartilage approximates to the cricoid, and the vocal cords are stretched and brought close. The epiglottis rises as the pitch rises.

When the upper limit of this register has been reached the tension on the various parts is extreme and one passes with relief to the middle register—the normal mechanism in the female (and tenors) for the production of notes between F_3 and F_4. The thyreoid cartilage returns to its normal position, the tension on the cords is decreased and they vibrate at their *thin* membranous edges only. As the pitch rises the thyreoid and the cricoid cartilages are again pulled together by the action of the crico-thyreoid muscle, and this state of tension lasts in tenors, sopranos and contraltos alike from F_3 to C_4. Higher notes than this are attained by a shortening of the vocal chink. In the small- or head-register the notes are produced by vibrations of only the inner margins of the cords, and the vocal chink is reduced to a *small* anterior aperture which becomes smaller as the pitch rises. These different mechanisms produce tones of perceptibly different quality (see Ear, Chap. XX.).

If the intensity ranges of the ear are again referred to (Chap. XX.) it will be seen that while the range of intensities covered by the human ear is large, covering frequencies from 32 to 16,000 vibrations per second, it is particularly sensitive to those frequencies lying between 1,000 and 5,000 d.v. per second. In this middle region it can pick up a pressure variation as small as one-thousand-millionth of an atmosphere. Now, frequencies of this order occur in phonation only in overtones. The main energy of the voice is of much lower pitch. The male voice has a very pronounced low component of about 120 d.v. per second, while the female voice has one about an octave higher. About 60 per cent. of the energy of the air emitted is due to vibrations having a frequency of less than 600 d.v. per second. Under 5 per cent. of the energy is associated with the production of overtones having a frequency of

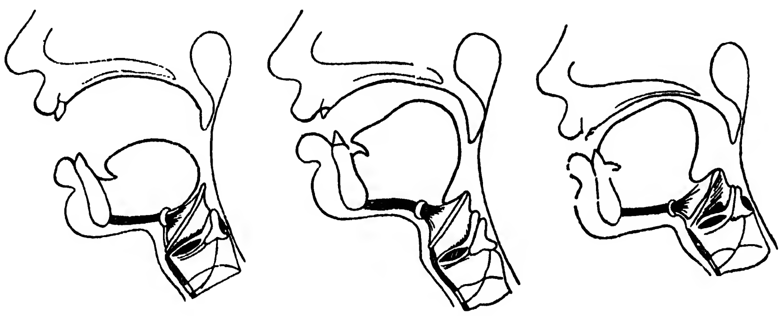
2,000 d.v. and over per second. We will return to this in dealing with speech.

Timbre or quality of the voice depends largely on the accessory resonating chambers. These cavities pick out and accentuate the overtones produced by the vibrations of the segments of the cords. Trained singers consciously or instinctively adapt the shape of the mouth so as to secure for each tone the most suitable overtones.

II. **Articulate speech** may be considered as the resultant of essentially two component factors, (*a*) the production of sound, and (*b*) the modification of the sound to produce speech.

Speech sound-units may be classed as vowels and consonants.

The vowels U, O, A, E and I are produced by the continuous issue of a blast of air through the mouth. **U, O** and **A**, pro-



A (*ah*) **U** (*ōō*) **I** (*ēē*)

FIG. 96.—Changes in the Shape of the Mouth in Sounding the Vowels, A, U, and I. (Grützner.)

nounced ōō (cook), oh and ah, respectively, are simple tones. They are produced by a regular series of vibrations emitted by a single cavity formed by lips, cheeks, palate and tongue (Fig. 96). This cavity is widest and shortest with **A**, longest and narrowest with **U**, while **O** is intermediate. On the other hand, **E** (as in pet) and **I** (= ēē) are double-toned. The back of the tongue is brought up against the front part of the soft palate so that the mouth is divided into two resonating cavities each with a characteristic note (Fig. 96).

By whispering the vowels one may readily determine the resonance-pitch characteristic of each. **U** has the lowest pitch, followed by **O** and **A**. It is thus easier to sing **U** and **O** on low than on high notes. An attempt to go up the scale by sounding " oos " will cause a tendency to clip the full vowel and sound a short " ee." The characteristic notes of each of these vowels (by percussion, Expt. 72, p. 556) is given by Helmholtz as follows (Fig. 97) :

The English **I** is really a diphthong and is pronounced by rapidly uttering the component unit sounds, *e.g.* **I** (as in fight) = **AI** = ah-ee.

Consonants are not continuous, but are sharply interrupted sounds. The issuing air is suddenly shut off by the lips to give the labials; by the teeth to produce dentals; by the tongue to give rise to gutturals. If the check occurs before the sound is produced, and the air is suddenly released, explosives are the result. The characteristic sounds of some consonants, *e.g.* **M** and **N** (which are mechanically the same as **B** and **D**), are produced by keeping patent the posterior opening of the nares. In this

$\mathrm{U} = F$
$\mathrm{O} = B^{\mathrm{I}}$
$\mathrm{A} = B^{\mathrm{II}}$
$\mathrm{E} = F^{\mathrm{I}}, B^{\mathrm{III}}$
$\mathrm{I} = F, D^{\mathrm{IV}}$

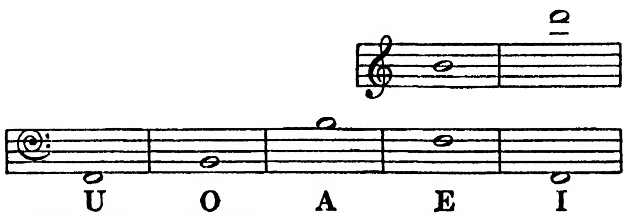


FIG. 97 (Starling).—Values obtained by percussing mouth cavity while shaped for the pronunciation of the vowels.

way some of the air comes continuously through the resonant nasal passages.

TABLE LXIII

CONSONANTS

Class	Labial.	Dental.	Guttural.
Explosives	P. B. V.	T. D.	K. G.
Aspirates	F.	S. L. Th.	Ch. (Scots)
Vibratives	—	—	R. (Scots)
Nasals	M.	N.	Ng.

All these with the exception of the hard consonants (*e.g.* **B** and **D**) can be pronounced quite well without the use of the larynx. The hard consonants are accompanied by phonation. Thus, although in pronouncing **D** there is a check at the teeth, the production of the laryngeal sound goes on.

The work done in speaking and in singing is complex. Many muscles are brought into play and the energy expended by them varies with the rate of speech and the intensity of the sound produced. The pressure of air employed in ordinary quiet conversation amounts to between 140 and 240 mm. of H_2O, while nearly 1,000 mm. are required when shouting is indulged in.

If *work* (phonation only) is taken as equal to the product of the pressure and volume of air expelled, *i.e.* **W = VH**, we may make a rough assessment of the amount of work done. During ordinary conversation, a man with a tracheal cannula developed an air pressure of 200 mm. (H_2O) and expired 300 litres of air per hour, *i.e.*

$$VH = \frac{300 \times 200}{1{,}000} = 60 \text{ kilogram-metres} = 0{\cdot}14 \text{ Cals. per hour.}$$

Speaking in a large hall the same man expired 1,440 litres of air per hour and developed a mean pressure of 700 mm. (H_2O).

$$W = 1{,}440 \times 0{\cdot}7 = 1{,}008 \text{ kilogram-metres} = 2{\cdot}36 \text{ Cals. per hour.}$$

One may arrive at an estimate of the work of speaking by measuring the oxygen used during rest and during speech. Delivering an oration at the rate of 150 syllables a minute caused the consumption of 28·78 litres of oxygen per hour. Subtracting from this the amount used during a similar period of rest, viz. 16·96 litres, we find that 11·82 litres were used by the orator. In large calories this amounts to $11{\cdot}82 \times 4{\cdot}9 = 57{\cdot}9$ Cals. per hour.

The value just obtained is about 25 times as great as that of mere phonation. We must remember that the orator (and the subject of the experiment was a Frenchman too) uses many additional muscles in gesticulation, etc. A speech of an hour's duration may tax his entire powers. To add to his troubles he had to make himself heard in a large reverberating hall.

Experiments have been carried out to determine the most economical way of using the voice so as to obtain distinct enunciation and carrying power. During ordinary conversation in a quiet room the oscillatory pressure (R.M.S.) in the sound wave at 1 foot from the mouth appears to be about 1 dyne per cm.^{2}. This value may be decreased or increased by about ten times without appreciably decreasing distinctness. At the lower energy level some of the consonants are difficult to differentiate. We have seen that the main energy of the voice is, speaking broadly, of low pitch. If we filter off the 60 per cent. or so of vibration of lower frequency, the distinctness and carrying power of the voice is not impaired, but if we filter off the 5 per cent. of the higher frequencies we reduce the audibility of articulation by about 25 per cent. This applies especially to the dental consonants—the "*hiss*" sounds having components of very high frequency, even above 4,000 d.v. per second.

The rate of emission of sound energy for ordinary conversational speech is about 125 ergs per second; for public speech about

2,500 ergs per second; and for tub oratory probably five times that amount.

R. L. Jones (Amer. Telegraph Co.) considers that the energy actually emitted in speech is very small. He calculates that if a million persons were to talk steadily, and *the energy of their voices* were to be converted into heat, they would have to talk for an hour and a half to produce enough heat to boil half a pint of water.

Sabine, who experimented largely with organ pipes as his source of sound, found that an open diapason organ pipe at a wind pressure of 9·14 grams per sq. cm. emitted 1,400 to 32,000 ergs per second according to the pitch of the note.

Table LXIV. gives the relative values of the expenditure of energy in the utterance of a single perceptible note in various sizes of halls. The notes were produced by an artificial larynx (syren).

TABLE LXIV

Nature of Hall.	Tenor.	Baritone.	Bass.
Theatre	1·0	3	7
Church	4	6	42
Lecture room	1·4	4	12
Dining hall (bad acoustics) .	4	6	66

These figures, multiplied by 2×10^{-5}, give, in kilogram-metres per second, the minimum expenditure of energy necessary to make the sound perceptible in ten different parts of the hall.

In every case the possessor of a bass voice has to expend more energy than the baritone or tenor. Generally speaking, the tenor has least trouble in making himself audible, but instances may occur where, on account of its resonating qualities, a building may prove more suitable for a baritone than for the former.

CHAPTER XXIX

ALIMENTARY CANAL

"I receive the general food at first,
Which you do live upon; and fit it is,
Because I am the store-house, and the shop
Of the whole body: . . . .
. . . . . Though all at once cannot
See what I deliver out to each:
Yet I can make my audit up, that all
From me do back receive the flour of all,
And leave me but the bran."

SHAKESPEARE.

As has been indicated (Chap. XXII.) the non-gaseous imports are submitted to a certain amount of manufacture before being handed over to the inland transport service for transportation to the cells of the body. For instance, proteins have to be split into their constituent amino-acids, carbohydrates are broken down into monosaccharides, and fats undergo some change. In addition to these changes in molecular complexity and preceding them come, in many cases, changes of physical state. Most of our foodstuffs are solid or semi-solid, and in such a state are useless to the organism. Before they can be split into their constituent units they must be rendered soluble.

General. In brief, the function of the alimentary canal is to provide (1) a series of mills and factories where food may be comminuted and dissolved in water, (2) a series of factories for breaking down the dissolved foods into units which the organism is capable of absorbing, (3) a mechanism for absorbing these units, (4) a mechanism for eliminating the waste material, (5) a means of transport from one factory to another, and (6) an adequate control over these various processes so that all may be co-ordinated.

In structure, the alimentary canal is a tube passing longitudinally through the body, having anteriorly a voluntary mechanism for receiving and grinding food; intermediately, stations, not controlled by the will, for completely breaking down the food mass to convenient units and for absorbing the same, and posteriorly, a semi-voluntary mechanism for ejection of waste.

I. The mouth is the port of the alimentary transport system. First, by nose and eye the cargo is sighted and its nature estimated. Messages are sent inland, factories get busy and all is ready when

the ship reaches port (conditioned reflexes). By means of the taste buds on the tongue, the nature of the cargo is further ascertained and appropriate secretions from the salivary glands take place (reflex). Bitter or saline substances provoke a profuse secretion of watery saliva. Flesh is met by a secretion containing a large proportion of the lubricating material—mucin. Dry matter causes the flow of a thinner and more watery saliva than moist matter.

(*a*) **The functions of saliva** *in the mouth are purely mechanical* (par. (*c*) below). It acts as a lubricant: moistening the surfaces of the mouth and the passage from it; infiltrating the food mass and so necessitating the expenditure of less energy in milling the food; and finally covering the outside of the bolus with mucin, thus rendering deglutition easy. *Normally, saliva has no chemical action in the mouth.* It contains a diastatic enzyme, ptyalin, which, however, carries out its action on polysaccharides during the earlier period of digestion in the stomach (*q.v.*).

(*b*) **The tongue** is a mobile organ lying on the floor of the mouth. It consists mainly of a mass of muscles which are paired. Some of these muscles lie wholly within the tongue (intrinsic), and for the most part, by their contraction, give rise only to alterations in shape. The extrinsic muscles have their point of attachment outside the organ, and so are capable of causing alterations in position as well as in form.

Intrinsic Muscles.

1. Superior longitudinal, pulls tip upwards and decreases length of dorsum.
2. Inferior longitudinal, pulls tip downwards and inwards, *i.e.* curves dorsum.
3. Vertical, working in conjunction with the transverse they produce a concave surface on the dorsum. Acting alone a convex surface is produced.
4. Transverse.

Extrinsic Muscles.

1. Genio-glossus—downwards.
2. Hyo-glossus—backwards.
3. Chondro-glossus (not always present)—backwards and downwards.
4. Stylo-glossus—backwards and towards palate.
5. Palato-glossus—side to side—continuous with intrinsic transverse.

The tongue has a threefold duty to perform as a unit of this transport system—(α) working in conjunction with the lip-sphincter—*orbicularis oris*, and with the triangular and other muscles it acts as a suction-plunger; (β) during deglutition it

functions as a force plunger, and (γ) it forms with the cheeks an effective hopper during the mastication of food.

(*c*) **The lower jaw** is a horse-shoe shaped lever of the *third* order. The load is placed on the teeth, the fulcra are at the ends of the horse-shoe, where they articulate with the fixed upper jaw, while the power is applied at a point on either side between the teeth and the fulcrum. The lower jaw is pressed against the upper jaw by the action of the temporal, masseter and internal pterygoid muscles which act antagonistically to the mylo- and genio-hyoids, to the platysma and to the anterior belly of the digastric muscle. Nuts having a crushing point of about 400 kg. may be crushed by a direct thrust of the front teeth. The molars, lying as they do nearer the fulcra and further from the application of the power, may exert a direct pressure of about 550 kg. The employment of such pressures is rarely necessary on account of the previous treatment of the food (milling, cooking, etc.), and of the influence of saliva. Soft bread, for instance, is merely compressed by a pressure of 100 kg., but, after moistening with saliva, only a twentieth of this pressure is necessary to obtain a clean bite through.

The grinding operations of the molars (and of the incisors at times) are a compound motion made up of a side-to-side and a forwards-backwards motion. The former is produced by the action of the external pterygoids working in conjunction with the posterior fibres of the corresponding temporal muscle. The latter movement may be ascribed to the *forwards* pull of the external pterygoids and the *backwards* pull of the posterior fibres of the temporals.

This mill-like motion tears the food with a smaller exhibition of pressure than direct crushing. Cooked meat which could be crushed by the application of from 30 to 100 kg. pressure can be torn by a grinding movement when the pressure is only 2 to 5 kg.

In Chap. XVII. we saw how bone was formed in accordance with the stresses and strains upon it. It is, therefore, interesting to note that in proportion as the food is prepared by factory milling and cooking, in proportion, in fact, to the avoidance of the stimuli to growth furnished by incident stresses and strains, so the jaws of civilised men tend to become weak. In consequence, faces are elongated and narrow instead of short and round like those of primitive men.

II. The act of swallowing. The food, after being chewed, is collected on the surface of the tongue by the action of the *buccinator* and other voluntary muscles. The jaws are closed and the tip and sides of the tongue are pressed against the hard palate and

teeth. A rapid contraction of the *mylo-hyoid* muscles, which form a floor for the front portion of the mouth (*diaphragma oris*), pushes the tongue up against the hard palate. At the same time the *hyoglossus* pulls the tongue backwards and the bolus is shot towards the gullet. This closes the voluntary stage of deglutition.

Several other muscles come into play at this point. As will be seen from Fig. 62 (lower portion), just above the larynx is a busy crossing common to two routes. Gaseous food and gaseous excreta pass to and fro right across the track of the descending bolus. At the moment of swallowing, the nose-to-lung and lung-to-nose traffic is reflexly held up. Further, the escape of the food mass by either of these incorrect routes is prevented as follows :

(*a*) The oral pharynx is closed by the action of the pharyngo-palatini muscles, which form the posterior pillars of the fauces. The pharynx is thus drawn to a narrow cleft. Against this narrow opening the soft palate is pressed by the action of the *levator* and *tensor veli palatini* muscles. (*b*) The laryngeal aperture is kept closed by the action of the *crico-arytænoideus lateralis*, *arytænoidei*, and the *thyreoarytænoidei* muscles, which pull the *arytænoid* cartilages forwards against the back of the epiglottis. Accompanied by a quick downward motion of the tip of the epiglottis, the bolus is pushed over the back of this structure and is impelled into the gullet. The following table (LXV.) shows the time relations of the chief muscles engaged in deglutition (from Kronecker and Meltzer and others) :

TABLE LXV

Time from Commencement.	Interval (seconds).	Muscle Movement.	Duration of Contraction (seconds).
—	—	Mylohyoid	0·6
—	0·03	Deglutition apnœa starts	5–6
—	0·07	Elevation of larynx	0·8
0·3 sec.	0·2	Constriction of pharynx	1–2
—	0·9	1st section of œsophagus	2–2·5
3·0 sec.	1·8	2nd section of œsophagus	6–7
6·0 sec.	3·0	3rd section of œsophagus	About 10.

III. The stomach. By the impulse imparted to it at its entry into the gullet, aided generally by gravity and to a questionable extent by peristalsis, the bolus is forced down to the gateway of the stomach. This aperture, in common with the exit from the stomach, is guarded by a thick ring of visceral muscle. When

contracted, *i.e.* during the normal state of tonus, these sphincters prevent the too hurried passage of material along the alimentary canal and also prevent its regurgitation. They are not controlled by the will but by local nerve centres. By its weight, the bolus usually exerts sufficient pressure to cause the opening of the cardiac sphincter and gain admission to the stomach, in which it is locked by the operation of a local automatic arrangement.

The processes of digestion or splitting of the foodstuffs by enzymes now commence (Chap. X.). Polysaccharides are broken down to maltose by ptyalin and the native proteins first converted to metaprotein by the action of the hydrochloric acid of the gastric juice and reduced in size and complexity to the proteose stage by the action of pepsin.

Pre-pyloric Sphincter.

Even a casual examination of the stomach will show that it is divided into two parts, each with a distinct function. Wepfer (1679), Spallanzani, Haller and others observed that a transverse band of muscle formed a " potential " sphincter to the *antrum pylori* in various animals including man. " This band," writes Beaumont, the observer in the now classical St. Martin experiments, " is situated near the commencement of the more conically shaped part of the pyloric extremity, 3 or 4 in. from the smaller end. In attempting to pass a long glass thermometer tube through the aperture into the pyloric portion of the stomach, during the latter stages of digestion, a forcible contraction is first perceived at this point and the bulb is stopped. In a short time there is a gentle relaxation, when the bulb passes without difficulty and appears to be drawn quite forcibly for three or four inches towards the pyloric end. It is then released and forced back, or suffered to rise again ; at the same time giving to the bulb-tube a circular or rather spiral motion and frequently revolving it completely over." Other observers, using more modern methods involving bismuth feeding, etc., have confirmed these older findings, and have shown definitely that this constriction takes place during *normal* digestion in man, dividing the stomach into an upper, fundic part, which *may* be completely separated physically and functionally from the lower, *antrum pylori*. The upper or cardiac portion is a reservoir or hopper where the food pulp is stored for a short time without mixing. It is during its stay here that salivary digestion reaches its maximum. By the steady pressure of the walls in the cardia, the mushy mass is fed little by little through the throat of the hopper (prepyloric sphincter) into the lower or pyloric part of the stomach. By peristaltic contractions

of its walls, this pyloric section of the stomach mixes food and gastric juice most thoroughly. The acid of the juice aids peptic while inhibiting diastatic action. Cathcart showed that the prepyloric sphincter was controlled by the hydrogen ion concentration of the duodenum and of the pyloric part of the stomach. Acid entering the intestine undoubtedly causes constriction of the sphincter. While the *p*H of the pyloric portion of the stomach does not seem to effect the *closure* of the sphincter, the above worker demonstrated that a sufficient reduction of the H ion concentration brought about a rapid opening of the sphincter. The introduction of an extra alkaline juice, *e.g.* by regurgitation from the intestine, leads to a smart flow of acid chyme into the *antrum pylori.*

Pyloric Sphincter.

The rate of exit from the antrum is controlled by the hydrogen ion concentration of the duodenum. As long as the duodenal contents are markedly acid the pyloric sphincter remains firmly closed, and only opens to admit more acid chyme when its receptors are no longer stimulated by acid.

IV. The intestines have three functions to perform: (*a*) transporting, (*b*) mixing and digesting, (*c*) absorbing.

(*a*) Transporting. This is carried on by means of a series of peristaltic waves, *i.e.* a section of the muscular wall adjacent to the distal end of the food-mass undergoes relaxation while a corresponding proximal section contracts. This double wave of relaxation and contraction passes along the tube and acts as a piston with a central orifice. In this way, the chyme is passed along at the rate of about 1 inch a minute.

(*b*) These driving peristaltic waves are not the only movements of the intestinal musculature. While these movements are going on in some loops of the small intestine, in other loops the chyme is kneaded and its surface broken by the rhythmic segmented contractions of the circular muscles of the bowel. By this means (i.) the various digestive juices of the intestine are thoroughly mixed with the chyme, (ii.) fresh surfaces are exposed to the absorbing surfaces of the wall, and (iii.) the capillary blood-vessels of the lining membrane are compressed rhythmically, so helping to drive the blood laden with the products of digestive activity on to the liver, etc.

The work of digestion, begun in the mouth and stomach, is completed in the intestine. Carbohydrates are reduced to single sugars and proteins are broken down to amino acids, etc. In addition to this, the fats are attacked by lipase, which resolves

them into their component fatty acids and glycerol (or other alcohol). In this process, the bile salts, by lowering the surface tension at the fat-lipase interface, play an important part.

(*c*) Absorption seems to be a case of passage of material through a membrane (*q.v.*).

V. **Fæces.** The materials not absorbed by the intestine are eliminated by the rectum as the fæces. One suggestive physico-chemical fact about these excreta is the proportion of soap to mass in their make up. It has been found that, normally, fat forms approximately one-third of the fæcal mass (dry). About 10 per cent. of this fat is in the form of soap. This may be correlated with the water-holding power of soaps and with their lubricating properties. Somewhere about 80 per cent. of their contents is water. This is somewhat remarkable, as both water, fatty acids and soaps are readily absorbed from the gut. If one desires to reduce the water content, calcium is exhibited. As we have already seen (p. 107) calcium soaps are hard " dry " soaps. On the other hand, the addition of easily dissociated sodium and potassium salts leads to the formation of " softer " soaps and a marked increase in the water content of the fæces. It is noteworthy that the fat content (as soap) remains constant. That unabsorbed fat is an excellent fæcal lubricant is an axiom in present-day prescribing when mineral oil (liquid paraffin), which cannot be absorbed, is given to produce easy defæcation.

For the final discharge of the waste alimentary contents, a simple kind of " touch button " mechanism is provided. The act is initiated by a voluntary response (removal of inhibition) to the stimulus produced by the stretching of the muscular wall of the rectum by the fæces. When the pressure of the fæces in the rectum reaches a value of about 30 to 40 mm. Hg., there is a call to defæcate. If no response be made, the call is not repeated immediately, as the rectal walls relax and so lose their irritability to pressure. While the initiation is voluntary the act itself is purely reflex like the other movements of the intestine. The reflex contractions and relaxations are generally aided by voluntary contraction of all the muscles which will increase abdominal pressure.

From a physico-chemical standpoint practically nothing can be said of the mechanism of alimentary transport. While the movements, etc., are apparent the underlying causes are completely hidden. No help so far is given by attempting to trace the development of the highly complex system of the vertebrate from the apparently simple physico-chemical response of the amœba to contact with food (Tropisms, Chap. XXXIII.).

Secretion of Acid and of Alkali.

According to Carlson, the total amount of gastric juice secreted by a man with a gastric fistula (on an ordinary diet of meat, bread, vegetables, coffee or milk and fruit) is about 700 c.c. per meal. Somewhere about 2,500 c.c. of water with sufficient hydrochloric acid to make about a 0·03 N solution is secreted per day. This concentration of a mineral acid is greater than is apparently compatible with life, and yet it exists in contact with the stomach wall. Further, the blood from which the acid undoubtedly comes is practically neutral (pH = 7·4). How, then, do the cells of the acid-secreting glands cause the separation of this *strong* acid from its salts and, having made the acid free, how do they maintain their integrity ?

(1) *Where is the acid formed ?* After intravenous injection of solutions of potassium ferrocyanide and of some *inert* salt of iron which will combine with the ferrocyanide to form Prussian blue only in the presence of *free* mineral acid, a blue colour is found in certain of the parietal cells. This seems to point to the presence of free mineral acid in these cells, but unfortunately for this thesis, some cells known to be secreting HCl, do not stain, while others incapable of forming HCl (*e.g.* liver, blood cells, etc.) do stain.

(2) *How is the acid formed ?* The present view is that the protein chlorides of the blood are dissociated slightly, as we have seen. Some of the free chlorions pass into the parietal cells and are seized upon by a weak base like ammonium. This weak chloride is secreted by the cells into the lumen of the gland where, meeting water, it is dissociated on the cell-lumen interface. The weak base passes back into the gland cells, and the strong $[Cl]^-$ unites with some $[H]^+$ from the water and passes into the stomach as HCl. It is known that cells, in contact with NH_4Cl and H_2O, can carry out such a chemical shuffling. For example, the mould *Penicillium glaucum* readily absorbs the ammonia from a solution of ammonium chloride, leaving hydrochloric acid. Evidence in support of this view is afforded by chemical analyses of tissues which show that while all contain ammonium salts those of the mucosa of the stomach contain the highest percentage. We have also seen (Chap. XXIII.) that there exists in blood a definite ratio between [protein HCl] and [NaCl], so that any decrease in the [protein HCl] will lead to a dissociation of [NaCl] to preserve the balance (see also Donnan Equilibrium, Chap. XI.). A disturbance like this has far-reaching effects (see alignment chart, Fig. 83).

When HCl is secreted there will remain an excess of base in the blood, hence, the denominator of the ratio $H_2CO_3/NaHCO_3$ tends

to rise and CO_2 has to be retained to preserve the balance at 1/20 But in doing so, the $[H_2CO_3]$ rises, and this will cause the alveolar CO_2 tension to rise. Dodds has shown that three-quarters of an hour after an average meal the alveolar CO_2 tension may rise by as much as 5 mm. Hg. In hyperchlorhydria the excessive withdrawal of $[Cl]^-$ from the blood leads to over double this increase of alveolar CO_2 tension. Those suffering from a low concentration of HCl in their gastric juice have a very slight increase in the output of CO_2, while achlorhydrics show no change.

The same problem, that of the secretion of alkali at a later stage of digestion, may be tackled in a similar way. It has been shown by Dodds that during the secretion of the alkaline juices (succus entericus, pancreatic juice and bile) the base of the blood suffers a decrease with a consequent fall in alveolar CO_2 tension.

Whatever be the mechanism by which these juices are formed, the epithelial cells can remain in contact with a considerable concentration of $[H^+]$ or of $[OH]^-$ without damage. Lowering of vitality, by anæmia, HCN, etc., permits digestion of the walls of the alimentary canal to take place.

CHAPTER XXX

MOVEMENTS OF THE LIMBS

" If the mountain will not come to Mohammed,
Mohammed must go to the mountain."

It is so obviously to the benefit of the organism to have the power rapidly to change its position relative to its prey and to those elements in its environment not in accordance with its comfort, that we take for granted that the process of the evolution of the means of locomotion is both natural and beneficial, and do not pause to consider how alterations in the external medium impressed the first organ of movement on gradually differentiating protoplasm. We refrain from embarking on this fascinating and highly speculative theme and leave to the student's imagination the progress from the surface tension changes occurring in the protrusion of pseudopodia; through movement produced like that of the "rocket car" by a backward expulsion of fluid; to the controlled ciliary whipping progression of free swimming *paramecium*. Then come probably problems of the laying down of fibrous tissue (*q.v.*) and the deposition of salts of lime, silica, etc., in this medium, forming a pattern such as we have seen on Chladni's plates (*q.v.*). The lever is an essential tool whereby muscle may be caused to do external work. Some animals lay down the solid mineral matter outside the limbs forming an exoskeleton. They have certain advantages in the matter of autotomy, but the disadvantages due to clumsiness and to the upheaval necessary to accommodate the growing bulk of the limb clearly outweigh these. The mammalian limbs contain their levers within, and the limbs carry the muscles outside the system of levers. We have already studied the structure of the levers (Chap. XVII.) and the intimate nature of muscular action (Chap. XIV.). We must now give consideration to the mechanism of the lever system of the body.

In order to get food, prepare food, and preserve its life and that of its race, the higher animal makes use of a series of levers to move its body in whole or in part. These levers are generally, but not always, made of bone, and generally, but not always, they work against a bony fulcrum.

In general, a lever is a rigid bar either straight or curved which is capable of a rotatory motion round a fixed point—the fulcrum. It is usual to divide levers into three classes depending on the relative positions of power, fulcrum and load.

Class I. The fulcrum lies between the point of application of the power and that of the load. In this class of lever, if the power arm is equal to the load arm, we have a balance. The application of 1 kg. of power will lift 1 kg. of load. If the power arm is lengthened by shifting the fulcrum nearer to the load, *then power will be increased proportionally as speed is decreased.* For example, dealing with a straight lever and putting P = point of application of power, F = fulcrum and L = point of application of load, then PF represents the length of the power arm, and LF = length of the load arm of the lever. If $PF = 10$ times LF, then 1 kg. at P would balance 10 kg. at L, *i.e.* the load of 10 kg. would be lifted by the exertion of a little over 1 kg. weight. This is the crowbar lever and is very little employed in the body. The most notable example of it is the forwards and downwards movement of the head when one is overtaken by unconsciousness, *e.g.* the nod of sleep. The fulcrum on which the head moves is the atlas, and the weight of the prefulcral part of the head (long power-arm) outbalances the postfulcral portion (short load-arm).

Generally, *speed is the desideratum.* The fulcrum is placed near the power. The power-arm PF is short and the load-arm LF is long. The relative speeds of the points will be as LF/PF. The catapults employed by the ancients to cast stones are examples of this kind of lever. The arm is used as a lever of the first class with a short power member when a cricket ball is thrown.

Normally, the head is a lever of this order, the power being applied very close to the fulcrum. The quick nod of assent is caused by the contraction of the anterior straight muscles which are yoked close to the fulcrum, while the slower backward movement is due to the placing of the effective muscles (splenii and complexi) somewhat further away from the occipito-atlantal joint. The feature of this arrangement is *stability.* Another good example is when the foot is lifted off the ground and the ground pressed on by the toes on contraction of the gastrocnemius.

Class II. The fulcrum is at one end of the lever, and the load lies between it and the power. That is, the power-arm is always of the same length while the load-arm may vary in length with the position of the load, *e.g.* nut-crackers. The outstanding example of this lever in the body is the foot. On rising on the toes, the base of the metatarsals is the fulcrum, the body-weight, borne by the tibia to the ankle, is the load, while the power is applied to

the os calcis by the gastrocnemius. A foot with a long load-arm, *i.e.* with the load near the power, is designed for speed not power—well adapted for running. On the other hand, the further the load is from the point of application of power, in this case, the longer the heel, the smaller will be the force necessary to lift the body. That this is so, may be inferred from a study of the development of the gastrocnemius muscle compared with the length of the heel bone. Europeans have short heel-bones and well-developed, bulky calves, while Africans have long heels and ill-developed calf muscles.

Class III. The point of application of the power is between fulcrum and load. This power must always be greater than the load. It is the commonest class of lever in the body, and this is to be expected, as its use results in the most rapid action possible. Speed is obtained, as before, by shortening the power-arm. In the arm, the Brachialis muscle is inserted about 1 cm. beyond the fulcrum (elbow), while the total length of the load-arm (fore-arm) is about 30 cm. The result of this arrangement is that the load (hand) moves with about 30 times the speed of the bone at the point of the application of the power. "Speed is gained at the expense of power." It follows that while a long-armed man may be able to give a quick blow he will be quite unable, unless his brachial muscles are abnormally developed, to give a heavy one.

This introduces a point to which the author of the "Tarzan" stories paid little attention. Tarzan was able to hold his own among the tree tops. Now, man has a fore-arm considerably shorter than the upper arm while the anthropoid ape has a fore-arm only a little short of twice as long as its humerus. This gives it a long and quick reach. In swinging and climbing, the upper arm is the lever employed to lift the body, mainly by the contraction of the Brachialis muscle; and the origin of the Brachialis over half-way up the humerus from the elbow (fulcrum) gives a power-arm with rather more power than speed. That is, a short humerus is a necessity for climbing animals—to furnish strength, just as the long forearm is necessary to give agility. To have equal climbing power, man would need to have extraordinarily bulky Biceps, etc., and this would not aid him when he desired to swing and seize distant branches surely and rapidly.

So far, we have dealt with the levers of the body in a general sense, as if they were straight bars. As a matter of fact, none of the bones of the body can be considered as straight levers, and none of the muscles act absolutely at right angles to the length of the bone. The length of the *effective* power and load arms may be

obtained by dropping perpendiculars from the fulcrum to the lines of application of power and load. The ratio of these perpendiculars gives the ratio of the distribution of power and speed by the lever.

The value of the bone-muscle mechanism depends on the mass of active muscular fibres, their degree of contraction and the angle which they make with the bone to be moved. The very movement of the bone will alter the angle of pull of the muscle. For each of its positions, the lever will have a *moment of rotation* determined by the size of the angle made by the line of traction (axis) of the muscle and the axis of the bone. By resolving the force of the muscle into two components, one of which acts along the axis of the bone and the other at right angles to it, one can readily perceive that the latter, the *effective component*, varies in value directly with the sine of the angle of pull. The ineffective or parallel component varies as the cosine of the angle of pull and represents the pressure exerted by the muscle on the fulcrum. As the moment of rotation is equal to the tension developed (F), and the perpendicular distance (d) of the axis of the muscle from the fulcrum, one may write $M = Fd$. Then the effective component is equal to $F \sin a$ where a is the angle of pull, and the parallel component to $F \cos a$. Hence, as the bony lever gets pulled up, the effective component will become greater and the parallel component will become less. In other words, the more parallel the axis of a muscle is to the axis of the bone which it is to move, the weaker will be its action—the maximum value is obtained when the line of action is at right angles to the bone.

Pulleys. By means of a single fixed pulley the direction of a force is altered, but not its magnitude. In the body, instead of reducing friction by means of a rotating pulley the tendon operates in a synovial sheath (*q.v.*). Good examples of the pulley may be found in the cartilaginous loop (*trochlea*) for the tendon of the superior oblique muscle on its way to the eyeball: and the peroneus longus looping round the lateral malleolus on its passage to the medial side of the foot.

Opponents. All the muscles attached to levers in the body are set in opposing pairs or antagonistic groups. As one group contracts, the opposing group will relax to exactly the same degree. The ulna, for instance, is pulled up towards the humerus by the action of the Brachialis, and it is pulled downwards by gravity and the action of the Triceps brachii muscle. Both sets of muscles act together and harmoniously, so that in any position of the ulna relative to the humerus, the opposing (muscular and gravity) forces exactly balance one another. That is, the

arm may come to any position and remain there without the expenditure of any extra energy (not taking into account gravity).

Synergists. Movement does not usually take place merely by the contraction of a muscle and the relaxation of its opponent. There are numerous other muscles brought into play, synergists—whose action, though secondary, helps the primary movements, generally by altering the pose of the body as a whole, but sometimes by immobilising the bone to which the muscle is fixed. As an example of the former, may be cited the action of the trunk muscles holding the body erect while a weight is being held above the head. The latter synergetical complex may be illustrated by the various muscles brought into action in opening a table drawer. " One hooks his fingers into the handle of the drawer and if it opens easily enough, the contraction of the flexors of the fingers is sufficient. If it works a little harder the flexors at the elbow contract to hold the bones of the forearm up so that the flexors of the fingers may have a firm origin. If still more force be needed the latissimus and teres major spring into action to support the humerus and rhomboids to hold the scapula. To make a strong pull one pushes against the table with the other arm and brings the extensors of the trunk into action, and finally if this does not suffice, the legs are braced and the whole body is converted by muscular action into a single solid piece in order that the flexors of the fingers may exert all their power to open the drawer." This description by Bowen shows clearly the complexity of an apparently simple action. The student will note too that as more muscles are called upon, the lever is lengthened and the position of the fulcrum altered.

Centre of Gravity. One of the first problems to be tackled is the maintenance of the body in a vertical position in a state of *stable* equilibrium. To produce this state of affairs the body must be balanced over the two feet so that the centre of gravity lies vertically over the area between the feet. In this position the centre of gravity of the whole body lies just above and half-way between the *anterior superior iliac spines.* As the body is bent forwards, backwards, or sideways the centre of gravity moves accordingly, and may lie outside of the body. As long as the vertical dropped from the centre of gravity lies within the area between the feet (underpropping area) the body will be in equilibrium. The maintenance of this balance is a function of the skeletal muscles. Whenever the centre of gravity tends to move beyond the underpropping area muscular contraction pulls it back.

Standing. In order to maintain a vertical position the *tibia* has to be balanced on the ankle-joint, the *femur* on the knee-joint, and

the *pelvis* on the hip-joint. Just try to balance an articulated skeleton on its two feet and you will get some idea of the complexity of the process. Each foot has three points of contact with the ground, viz. heel, base of hallux, and varying proportions of the outer digits. Thus, the body is balanced on two tripods (Fig. 52). These tripods can be placed in various positions relative to one another so as to spread the area of support and keep the plumb from the centre of gravity within it. With the feet in the position adopted when one stands erect and at ease, one has got to place the tibiæ on the tripods so that the centre of gravity of the parts superior to the ankle-joint lies as nearly as possible over the axis of the joint. Having done this, we have, in a similar way to balance the femur, and then the pelvis with vertebral column, thoracic cage and arms. Finally, the head has to be placed on the atlas.

Bending. The further the vertical from the centre of gravity moves from this " at ease " position, the greater is the difficulty of maintaining equilibrium. More muscles are called into play and they have to bear a greater load. Fatigue, which we have seen is rapidly produced by continuous exertion, soon sets in.

In bending forward the centre of gravity passes forward out of the body and lies well in front of it. To counterbalance this, the hips are thrust back and the body assumes a > position. If this is not done, we would fall forward, as any one may find out if he tries to pick from the floor a coin placed between his feet, protrusion to the posterior being prevented by standing against a wall. This function of the hip movement is apparent also when one tries to stand on one foot. On shifting from both feet to one foot, the hips shift towards that side on which the foot is being used, and compensatory movements of the shoulders and other parts occur. If one stands vertically upright, heels against a wall which prevents the backward movement of the hip, it is just as impossible to stand on one foot as it is to touch the toes without falling.

Walking. The process of walking is a series of acts whereby a state of unstable equilibrium is first produced, and then corrected by altering the position of the underpropping area of the body. It might be described as falling forward followed by recovery. It has been extensively studied by Hill and others by means of the ultra-rapid cinematograph camera producing the so-called " slow-motion " pictures. These records show that, from the standing position, natural walking is accomplished by leaning forward and when the angle of slant assumes a critical value indirectly related to the height of the walker's centre of gravity from the ground, one foot is raised, swung forward, and its heel placed on the ground.

The body then falls forwards and towards that side on which the foot has moved. This brings the centre of gravity over that foot, and to prevent undue muscular action this leg is straightened, bringing the centre of gravity right over the ankle-joint, etc., as already explained. The other foot aids this process of the redistribution of the incidence of the weight of the body by pushing against the ground with its toes and raising the heel from the ground to do so. The second foot is now raised clear of the ground by flexion at the knee and lifting of the toes. This foot follows a similar path to the first foot, *i.e.* is swung forward by muscular contraction until it reaches a position where the heel is placed on the ground by the extension of the knee.

The leg thus acts, in walking, like a compound pendulum, and, therefore, the amplitude of its swing, *i.e* the length of the stride, will be determined by the length of the pendulum, *i.e.* by the distance between the centre of gravity of the limb and the hip-joint. A short leg will, therefore, have a short quick swing, and a long leg a long slow one. By altering the effective length of the pendulum by lowering or raising the centre of gravity of the limb one produces an amplitude of swing to which the leg muscles are not accustomed. Fatigue will in these circumstances set in more rapidly than normally. The alteration in the centre of gravity may be accomplished quite simply by wearing extra heavy or extra light-weight shoes.

As was mentioned above, the hip moves backwards to compensate the shift forwards of the centre of gravity. One would, therefore, expect to find a compensatory movement of the hips in walking in order to keep the centre of gravity near the inner border of each foot in turn. Not only does this side-to-side movement take place, but two other hip movements occur which together cause the hips to follow the line of a double sigmoid curve with each complete step. (1) When the body is falling forward the hip moves in the arc of a circle, of which the centre is the ankle-joint and the radius is the distance between hip and ankle. (2) During the period when the weight of the body is being shifted to the other leg the hips trace the arc of a circle whose radius is half the distance of foot to ground.

Owing to the shortness of the *ileo-femoral ligament* in the female, her leg cannot swing back as far as that of the male. If she wishes to lengthen her stride to keep pace with a male partner, she must twist her pelvis on the vertebral column. Because of the ileo-femoral ligament and the wide pelvis, the adult female has a gait characterised by a greater degree of rotation than that of the male or of the younger female.

SECTION V.: THE ANIMAL AS A WHOLE

CHAPTER XXXI

THE PRESERVATION OF NEUTRALITY

"To test a principle by its consequences is allowed by good logic and enjoined by sound reason." JOUBERT.

FROM a physico-chemical standpoint, the animal body may be considered as a polyphase liquid, the various phases being separated from one another by a series of membranes of varying and variable permeability. From the moment of origin of the organism as an entity, that is, from the time when the conjugation of spermatozoön and ovum produced a mass of protoplasm which was not in equilibrium with its environment, the various external forces brought to bear on the organism still further accentuate this disturbance. The sum of the changes termed life may be looked on as the response of this polyphase-multimembrane-enclosed liquid to these impacts. Briefly, all changes tend to restore balance—which, when attained, is death. It is difficult to make statements on this subject without using terms implying purpose, for example, we speak of water reaching its own level—cell adjusting itself to its environment, etc. To use other terminology would be cumbrous if correct. So, in discussing the relationship existing between cell and environment, individual and world, etc., we find it convenient to consider how the organism so adjusts itself to meet changes in its environment that it remains apparently an independent entity. There is, of course, no such somatic independence. Body and environment together are in indissoluble partnership. In fact, they are a unity. The organism no more adjusts itself to suit its environment than the environment alters itself to suit the organism. Both are subject to change, but the change is equally impressed on both.

Further, a change in the nature or incidence of any force on organism or environment produces far-reaching results. Every unit of the entire system (organism + environment) is affected more or less by quite a small change in the energy content applied apparently locally. For example, we dealt (Chap. XVII.) with the

effect produced, on the internal and external structure of bone, of altering the incidence of a load. Not only are the nearby bones altered, but even distant and apparently unaffected bones undergo changes. In our study of the transport system we saw how an alteration produced in one part of an organism spread throughout the whole animal.

One of the main functions of blood is to maintain constant the concentration of hydrogen ions throughout the organism. A slight potential increase in the acidity or alkalinity of the system acts as a trigger, setting off a series of reactions resulting, finally, in the restoration of the *status quo*. Acid is set free, say in muscle, and before it can be rebuilt into the muscle complex, oxidation of glucose has to take place. The introduction of this acid in a minute amount has three profound effects. Firstly, it increases the dissociation of oxyhæmoglobin (p. 328) setting free the needed quota of oxygen, secondly, by stimulating the respiratory centre (Chap. XXVI.), it speeds up the intake of oxygen, and lastly, the blood flow is increased, both *locally* by vasodilatation, and *generally* by increased cardiac action.

In the work of preserving the neutrality of the organism the blood is aided by the eliminating organs—the lungs and the kidneys.

Factors Tending to Preserve Neutrality.

I. In the plasma we have (*a*) colloids, and (*b*) crystalloids.

(*a*) The colloids of blood plasma are mainly serum albumin and serum globulin, and they are amphoteric in character, *i.e.* they may act either as acids or as bases. Experiments carried out in the laboratory show definitely that, although the proteins of the plasma readily combine with mineral acids, they are unable to react with the weakly dissociated acids found in the body. Both albumin and globulin form hydrochlorides for instance, but protein lactates and carbonates are unknown. At the hydrogen ion concentration of blood, however, the proteins may act as weak acids and combine with some of the plasma base. When carbon-dioxide passes into the plasma it reacts with the protein salt, liberating the protein as a weak acid, and forming sodium bicarbonate with the base. The weak protein acid is so slightly dissociated as to have a negligible influence on the hydrogen ion concentration.

Some carbon-dioxide may also be absorbed by the colloidal particles, but the part played by the serum proteins in the preservation of neutrality is quite small.

(*b*) As has been stated, the main crystalloids of the plasma are sodium bicarbonate and sodium chloride, but there are small

quantities of other salts such as phosphates also present. These latter are, however, quantitatively unimportant. In the regulation of the hydrogen ion concentration, the sodium bicarbonate is the most important plasma constituent. It has been shown practically, and it may be deduced theoretically, by consideration of the law of mass action, that where a solution contains a weak acid, and a salt of that acid, the hydrogen ion concentration of the solution is proportional to the ratio $\frac{[\text{concentration of free acid}]}{[\text{concentration of salt}]}$.

Of the crystalloids in solution in the plasma, sodium bicarbonate has the most marked "*buffer*" effect. In this salt a strong base is united with a weak acid and, therefore, any acid stronger than H_2CO_3 will take the place of the HCO_3 ion in its combination with sodium.

For instance,

$$\begin{array}{ccccl} \begin{array}{|c|}\hline Na \\ CH_3COO \\ \hline\end{array} & \begin{array}{c} + \\ + \end{array} & \begin{array}{|c|}\hline HCO_3 \\ H \\ \hline\end{array} & \begin{array}{l} = \text{Sodium bicarbonate.} \\ = \text{Acetic acid.} \end{array} \\ \| & & \| & \\ CH_3COONa & & H_2CO_3 & \\ \text{(Sodium acetate).} & & \text{(Carbonic acid).} & \end{array}$$

This reaction appears just to postpone matters because carbonic acid is set free and, although this acid is only slightly dissociated, yet, as an acid, it must be reckoned with. The dissociation of $NaHCO_3$ is a reversible reaction :

$$\begin{array}{lcccc} \begin{array}{l} NaHCO_3 \\ H_2O \end{array} & \begin{array}{c} \rightleftharpoons \\ \rightleftharpoons \end{array} & \begin{array}{|c|}\hline Na^+ \\ OH^- \\ \hline\end{array} & \begin{array}{c} + \\ + \end{array} & \begin{array}{|c|}\hline HCO_3^- \\ H^+ \\ \hline\end{array} \\ & & \text{Strong.} & & \text{Weak.} \end{array}$$

This means that the direction of the reaction will, in the main, depend on the relative amount of $CO_2(H_2O)$ present compared with the amount of $NaHCO_3$. For plasma, this ratio has been determined experimentally. To give the normal pH of 7·4, plasma must have 3·75 grams of CO_2 bound in $NaHCO_3$ present for every gram of uncombined CO_2: or, by volume, one volume of CO_2 remains constant when associated with 20 volumes combined as carbonate. In tabular form this reads :

$$\frac{\text{Free } CO_2}{\text{Bound } CO_2} = \frac{1}{3{\cdot}75} \text{ by weight, } \frac{1}{20} \text{ by volume.}$$

If excess of $NaHCO_3$ over this ratio is present owing to reduction in the CO_2 tension some will dissociate to balance the dissociation

pressure. If too much CO_2 is present it will in the first instance combine with any available base to form a bicarbonate; if no base is available, the excess CO_2 will be eliminated by the lungs.

If any acid stronger than carbonic acid finds its way into the blood it replaces some of the bound CO_2, thereby increasing the free CO_2 temporarily. This causes increased ventilation of the lungs and elimination of the excess CO_2, bringing the ratio $\frac{\text{free } CO_2}{\text{combined } CO_2}$ back to its original level, and hence restoring the original pH.

The bicarbonate of the plasma represents the excess of base which is left over after all the non-volatile acids have been neutralised. It is the **alkali reserve** of the body which can be drawn upon to neutralise any free acid stronger that CO_2 which may find its way into the blood stream (Demonstration, Part II.). Not until practically all the alkali reserve has been used up will the blood show any change in hydrogen ion concentration. Long before that point can be reached other mechanisms will be brought into action to preserve neutrality. *The bicarbonate is a nest egg of potential base which may be drawn upon when required, but the inroad must be made good at the first opportunity. It is really an emergency measure useful to tide one over the difficulties that occur suddenly and frequently. It is not a widow's cruse of oil—always magically replete.* If the ratio of H_2CO_3 to $NaHCO_3$ is kept within normal limits even though the reserve is permanently lowered, the acidosis necessitating the draft on the reserve is called *compensated.* If, on the other hand, the amount of H_2CO_3 increases to a value greater than 1/20 of the alkali reserve in arterial blood, the acidosis is said to be *uncompensated.*

II. The lungs eliminate CO_2. The amount eliminated per unit of time is a function of the capacity of the lungs and the rhythm of respiration. The rate and depth of respiration are controlled by the amount of CO_2 in the blood perfusing the respiratory centre in the medulla oblongata. Any increase in the CO_2 of the blood causes an increase in the rate of respiration. Similarly, the process of respiration may be slowed down, till it stops, by decreasing the amount of CO_2 in the blood. It has been stated that this regulatory action of the medulla is caused not by CO_2, but by the hydrogen ion concentration of the blood, *i.e.* any acid perfusate will quicken respiration. But, as is obvious from the context, *no free acid but* CO_2 can occur in the blood of a living animal. Further, careful research has shown that the pH of blood does not alter in health, so nice is the regulation.

III. Excess of base, and acids in combination with bases are eliminated by the kidney. The cells of this organ have a low threshold for such salts.

IV. *The part played by the red blood corpuscles in preserving neutrality.* In common with other tissue elements, the erythrocytes have a phosphate buffer system. Potassium dihydrogen phosphate, KH_2PO_4, is an acid salt and reacts with bicarbonate, for instance, to form the basic salt K_2HPO_4,

viz. : $\underset{\text{acid phosphate.}}{2KH_2PO_4} + 2NaHCO_3 \rightleftharpoons \underset{\text{basic phosphate.}}{Na_2HPO_4 + K_2HPO_4} + H_2O + CO_2$

A mixture of these two phosphates such as is found in all tissues obviously will not increase in acidity till nearly all the disodium phosphate has been converted into dihydrogen phosphate, nor will the [H]. markedly decrease till all the dihydrogen phosphate has been converted into the basic salt.

In addition to this, the pigment hæmoglobin plays a very important part in the preservation of neutrality, as might be deduced from its function in the transport of respiratory carbon-dioxide. As explained in the chapter on respiration, hæmoglobin, either in the reduced or oxygenated form, appears to be present in the cells either as a weak acid, or in combination with cell base as a salt. Increase in the concentration of acid in the cell due to increase in the acid in the plasma results in reactions of the type $BHb + HA \rightarrow BA + H \cdot Hb$, as long as the acid HA is a stronger acid than hæmoglobin. Acids weaker than hæmoglobin would have a negligible effect on the hydrogen ion concentration because of their slight dissociation. Thus in the presence of a stronger acid, base is liberated from combination with hæmoglobin to neutralise the stronger acid, while the resulting acid hæmoglobin has a minimal effect on the hydrogen ion concentration because of its low dissociation.

In addition to this "buffer" action of the hæmoglobin, in which it acts simply as a weak acid and salt buffer system like the free and bound carbon-dioxide of the plasma, the pigment has a still more important influence on the preservation of neutrality by virtue of its change in acidity on oxygenation as described in Chap. XXIII., whereby, without change in hydrogen ion concentration, the mere passage of hæmoglobin from oxygenated to reduced form renders available about 25 per cent. of base combined with it for combination with other acids. It is these physical and chemical properties of hæmoglobin, together with the re-distribution of electrolytes between cells and serum which accompanies the reactions between hæmoglobin and acids, which confer on blood a "buffering" power superior to that of plasma.

V. The tissues themselves exert a neutralising effect on the blood. As mentioned above, they are endowed with a phosphate buffer system.

To summarise :

Blood and tissue fluids normally neutral $p\mathrm{H} = 7{\cdot}4$.

Alterations caused.

1. Tending towards alkalinity—Alkaline tide after digestion.
2. Tending towards acidity.

A. Normal. (1) Muscular activity. CO_2.
Lactic acid.

(2) Protein disintegration. food / muscle $\left\{\begin{array}{l} H_2SO_4 \\ P_2O_5 \end{array}\right.$

B. Abnormal. Mal-oxidation acidosis.

Alterations checked.

1. Tissue compensation—Phosphates.
2. Alkali reserve—$NaHCO_3$.
3. Respiration $\left\{\begin{array}{l} CO_2 \text{ stimulates respiratory centre.} \\ \text{Lack of free } CO_2 \text{ depresses centre.} \end{array}\right.$
4. Kidney $\left\{\begin{array}{l} (a) \text{ increased elimination of } \left\langle \begin{array}{l}\text{acid} \\ \text{alkali}\end{array} \right\rangle \text{ as salts.} \\ (b)\ NH_3 \text{ salts. (Liver action.)} \end{array}\right.$

An apology is necessary for the use of the word "*buffer*" to denote the power of phosphates and bicarbonates to maintain a steady $p\mathrm{H}$ in spite of additions of acid or alkali. The late Professor Sir Wm. Bayliss has pointed out the misleading nature of this expression and has shown how it crept into use. Non-descriptive as the word undoubtedly is, it has found a place in current physiological and physico-chemical literature, dislodgment from which will be a difficult task.

CHAPTER XXXII

THE REGULATION OF TEMPERATURE

"Where hot and cold, and dry and wet
Strive each the other's place to get."
PRIOR.

THE problems associated with the regulation of the temperature of man are so closely connected physically and physiologically with those involved in the provision of an adequate ventilation in rooms, etc., that these two subjects may conveniently be considered together.

One of the most striking phenomena in the life of man and of the warm-blooded animals generally is the remarkable constancy of the temperature maintained in spite of the variations of temperature to which they may be subjected. This is a fact which did not escape the attention of the ancients, who thought out many weird and wonderful explanations. Even well on in the nineteenth century, text-books echoed the idea of Haller (1757) that animal heat arose mainly from the friction of the blood in the vessels.

The mammal or the bird may travel from the Arctic regions where the external temperature may be at $-53°$ C. to the tropics at 53° C. without much increase in body temperature. Contrast this freedom from variation with the continuously changing temperature of the cold-blooded animal as the temperature of its environment changes.

TABLE LXVI

Temp. of Water.	Temp. of Frog's Stomach.
2·8° C.	5·8° C.
20·6° C.	20·7° C.
41° C.	38° C.

Within natural limits, the temperature of the cold-blooded animal is usually about 1° C. above that of its environment. It is interesting to note that those animals which hibernate become, for that time, as if cold-blooded. The advantages that warm-

blooded animals possess by reason of their higher temperature are due to the well-known fact that most chemical and some physical reactions are increased in rate by increase of temperature (see Temperature Coefficient). They also are free from constant fluctuations of temperature. Against this must be placed the fact that they have to maintain a temperature greater than their environment by about 20° C.

Homoiothermic Animals maintain a constant temperature.		
Mammals and Birds . . .	Birds . . .	about 42° C.
Adult and not during hibernation or activity . . .	Mammals (except man)	,, 39° C.
	Man . .	,, 37° C.
Heterothermic or Poikilothermic Animals.		
(*a*) Lethal temperature, about 20° C.	New born Homoiothermes. The young of rats, mice and man are practically poikilothermic, and maintain an internal temperature of from 0·01 to 3·0° C. above the temperature of the environment.	
(*b*) Hibernation starts when temperature falls to about 20° C.	Hibernating animals.	
(*c*) Still active below 20° C.	Reptiles, Batracia, Fish, Molluscs, Insects, etc.	

Of all animals, birds have the highest temperature. For example, that of the chicken is about 43·8° C.: of mammals, the rabbit and the fox have a temperature as high as 40° C., while the horse and the elephant come low on the scale with 37·6° C.

In health, the temperature of the human body varies so little from the normal value of 37° C. (98·4° F.) that temperature is regularly taken as a clinical indicator and any fluctuation from the normal points to the employment of remedial measures.

Instruments Used to Indicate Animal Temperature.

(*a*) Mercury thermometer.
(*b*) Electrical resistance thermometers.
(*c*) Thermo-electric couple (Thermopile).

(*a*) The *thermometer* was probably invented by Galileo (1603), and was first used clinically by Sanctorius (1626), who reported the temperature of a fevered man.

The clinical thermometer is an adapted form of the common mercurial thermometer having, (1) a long cylindrical reservoir to admit of rapid attainment of equilibrium between body and mercury. (2) A small bulbous part just above the mercury reservoir to catch the mercury driven out of the reservoir by the expansion due to the increase in temperature to 34° C. (3) A small bore capillary graduated from 35° C. to 45° C. to admit of reading to a tenth of a degree, and finally, (4) another bulbous part to catch any

mercury that might be driven over by accidental heating beyond 46° C. Usually the thermometer is made self-registering by having a small detached thread of mercury which is pushed up by the expanding fluid and remains at the highest temperature reached.

Where a continuous record has to be made, or where great accuracy is desired, one of the electrical methods is employed.

(*b*) This method depends on the alteration of the *electrical resistance* of a platinum (or other metal) wire caused by a slight increase in its temperature. The alteration in conductivity may be measured by a Wheatstone's bridge or may be recorded photographically as in the study of the electrical changes in tissues (*q.v.*). This is by far the more sensitive of the two electrical methods, but great care must be exercised in its use. It has, however, some disadvantages. For example, the current flowing through the thermometer tends to heat the fine coil of wire. The heating is proportional to the square of the current, and to the resistance of the wire.

(*c*) *The thermopile* is based on the principle that, if the junction of two dissimilar metals (*e.g.* constantan and iron) be warmed, a difference of potential between the two will be produced. In order that small fluctuations of temperature may be measured, a second thermopile is arranged in the circuit in series with but opposed to the first. This second thermo-electric junction is kept at a certain known temperature differing but little from the temperature to be measured. By this means the bulk of the electromotive force resulting from thermopile 1 will be neutralised by the E.M.F. produced by thermopile 2, and thus the E.M.F. produced by a small change of temperature becomes a relatively large proportion of the net E.M.F. The current changes are read from a potentiometer.

Location of Thermometer.

(*a*) Natural Cavities.

(i.) Mouth. The cavity underneath the tongue is generally chosen by the physician as suitable for the taking of temperature. A half-minute thermometer should remain at least 3 minutes *in situ*. If the mouth is kept open, low readings will be obtained due to the inrush of cold air and the vaporisation of moisture. (Average value = 36·87° C.)

(ii.) Rectum. Practically all physiologists are agreed that the most favourable position for the thermometer for the indication of the true temperature of the interior of the body is the rectum (or vagina in females). The instrument should be inserted sufficiently deeply (7 cm.), to make sure of the maximum temperature, and, in the case of the rectum, it should not be imbedded in fæcal matter. (Average value = 37·2° C.)

(*b*) Artificial Cavities.

(iii.) Axilla. In the private practice of a physician, temperatures are generally taken from the armpit. Care has to be taken to free the skin from moisture and sweat and to close the cavity on the thermometer for a time sufficiently long to enable the surface of the skin to attain the temperature of the interior of the body. (Average value = 36·9° C.)

(iv.) Groin. In young children the thermometer is usually placed in the fold of the groin.

While the temperature of the body is so constant that it is taken as a clinical sign, yet it does vary regularly by about $\pm$ 0·5° C. during the 24 hour day. From the chart (Fig. 98) it

will be seen that between 3 and 4 o'clock in the morning body temperature is at its lowest and between 3 and 4 o'clock in the afternoon it is at its highest. This rhythm or periodicity is shown by night workers as well as by those who live a normal average life. After a very few days they become "acclimatised" to their

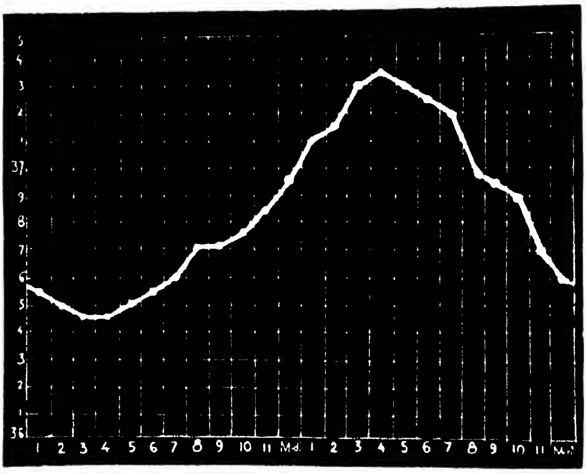


FIG. 98.—Chart of the Daily Variation of Temperature in the Normal Human Subject, in degrees Centigrade. The hours are marked from midnight. (Richet.)

reversed "day," and thereafter have their peak temperature at about 4 a.m. and their lowest temperature in the early afternoon. Some people adapt very slowly. For instance, Benedict tells of a night watchman who for seven years had been working during the night and sleeping during the day, and had his maximum temperature about 4 o'clock in the afternoon when sound asleep and his minimum about 4 o'clock in the morning when he was awake and active.

It is common knowledge that certain means may be taken to bring about alterations in the body temperature :

1. Muscular exercise causes heat.

2. Want of muscular exercise is followed by cooling, *e.g.* cold has to be guarded against during sleep.

3. Heat or work causes sweating (*q.v.*)

4. Heat produces lassitude. Compare the day's work of a man in Spain with that of a man in Scotland.

5. Still air has the same effect as that of heat on the feeling of lassitude. The production of a movement of the air removes the tired feeling.

6. A higher temperature can be endured in a Turkish than in a plunge bath. Further, a hot dry climate is comfortably endured, while air at the same temperature, but *saturated with moisture*, oppresses.

7. Cold air does not chill one to the same extent as water at the same

temperature. Cold much more severe than is experienced in Britain may be enjoyed in the dry, Canadian climate.

For example, a temperature of 40° may be borne if the air be dry and still, while a much warmer but damp and windy atmosphere may "cut to the bone."

8. Ingestion of hot or cold food or of heat-producing food normally produces an almost negligible variation in rectal temperature.

What we want to get at is the mechanism whereby the body temperature of the higher mammal is kept fairly constant despite variations of outside and "inside" temperature. In this respect, the ordinary laws of cooling-surface phenomena, are obeyed. Heat may be lost by:

I. Radiation.
II. Conduction and convection.
IIa. Ingestion and excretion. Inspiration and expiration.
III. Evaporation of moisture.

I. Radiation

The transmission of heat by radiation differs essentially from conduction and convection. The particles of a body have a vibratory movement depending on their kinetic energy. Increase of temperature will, therefore, cause increase in the velocity of these movements. In conduction, some portion of another body is heated by contact with the warm body. This second body passes the heat on, *i.e.* there is molecular continuity. By radiation, on the other hand, one body can affect the thermal state of another body not in contact with it without sensibly affecting that of the intervening medium. Radiation is not dependent upon the presence of air. It takes place quite readily in a vacuum. This is manifest when we consider how we derive heat from the sun whose radiant heat is transmitted through the ether at a very high velocity (about 186,000 miles per second) by means of transverse wave motion.

Radiant heat may be detected and measured by means of a suitable thermopile provided with a collecting horn or by means of Leslie's differential air thermometer. The amount of heat lost by radiation depends on:

(i) **The area and colour of the surface.** It has been proved experimentally as well as deduced mathematically that the emissive and absorptive powers of a body are equal. A perfectly black body absorbs all the heat energy that falls on it, and therefore, since a body cannot absorb more than is incident on it, the absorptive power of such a body is unity. The emissive power of a body is the ratio of the quantity of heat radiated per square centimetre

of surface to the quantity radiated per cm.^{2} of a "perfectly" black body under the same conditions. (Expt. 74, p. 556.)

TABLE LXVII

RADIATING AND ABSORBING POWERS OF BODIES

Lamp black	1·0
Cinnabar	0·28
White paper	0·9
White lead	0·21
Polished silver	0·03

(ii.) **Temperature of the surface.** There is a connection between the amount of radiation from, and the temperature of, a body. The total heat-loss by radiation is proportional to the difference between the fourth powers of the absolute temperatures of the body and its environment as well as to the area and nature of the surface. If body and environment differ little in temperature, the heat lost by radiation will thus be comparatively small. For example, if the temperature of the air were 17° C., the heat radiated from a "perfectly" black man at 37° C. would be $(273 + 37)^4 - (273 + 17)^4 = 310^4 - 290^4 = 216 \times 10^6$ per unit surface.

Of course a perfectly black man does not exist, nor yet a perfectly white man. If black skin has a radiation-coefficient of 0·98 and white skin of 0·7, then the difference in radiating power would be expressed by $\frac{0 \cdot 98}{0 \cdot 7} = 1 \cdot 4$, a trivial advantage to the black-skinned man in a tropical climate.

II. Conduction

By conduction is meant the loss of heat from a body at a higher temperature to one at a lower temperature by passage from particle to particle, for example, the passing of heat along a poker, one end of which is in the fire. The amount of heat lost by the body in this way depends on several factors.

(i.) **Surface Exposed.**

(*a*) **Area.** The loss of heat varies directly with the area of the surface exposed. For example, the flow across 2 sq. metres is double that across 1 sq. metre. The area of surface of a child of 2 years weighing 10 kg. is 5,120 sq. cm., and of a man of 60 kg. is 14,079 sq. cm. That is, the area per kg. for a child of 2 years is 512 sq. cm., and for a man is 234 sq. cm. It is obvious that weight for weight the child will lose more heat than the man.

(*b*) **Moistness.** The loss of heat depends on the moistness of the surface. The thermal conductivity of all liquids except mercury is very low. Water may be boiled in the top portion of a test tube without affecting a piece of ice at the bottom.

TABLE LXVIII

THERMAL CONDUCTIVITY

Substance.	Conductivity (c).	Substance.	Conductivity (c).
Silver	1·52	Cotton flock	4×10^{-5}
Platinum	$1·14 \times 10^{-1}$	Water at 37° C.	135×10^{-5}
Marble	180×10^{-5}	Sea water	108×10^{-5}
Wood	10×10^{-5}	Olive oil	39×10^{-5}
Paper	30×10^{-5}	Human skin	60×10^{-5}
Vulcanised rubber	$8·9 \times 10^{-5}$	Lard	48×10^{-5}
Wool	5×10^{-5}	Leather	42×10^{-5}
Compressed cotton	3×10^{-5}	Air (dry)	5×10^{-5}

Several measurements of the temperature inside the body have been made. The natural cavities, *e.g.* the rectum, and vagina, are generally used, both being deep cavities into which the thermometer or thermal elements can be inserted for a considerable distance. Measurements have been made of the temperature in the inside of the stomach in patients with gastric fistulæ. The temperature of freshly voided urine was suggested by Stephen Hales in 1731 as representing that of the interior of the body. It has been found that, while the temperature of the surface of the body is about 32° C., as the depth from the surface increases, the temperature rapidly rises till the depth of about 5 cm. is reached. It continues to rise much more slowly for the next 2 cm. or so. Apparently the temperature after this is fairly uniform, *i.e.* 37° C. That is, the subcutaneous tissues cause a temperature lag or thermal gradient of about 5° C. This is due to the large proportion of water which enters into the composition of the tissues, giving the body the high specific heat of about 0·83. A man weighing 60 kg. would consequently have a water equivalent of 50·4 kg. of water, so that a rise of 0·1° C. would only be registered after 50·4 Cals. of heat had been rendered latent.

(*c*) **Fat.** The loss of heat depends on the nature of the surface and of the subcutaneous tissue. A good layer of subcutaneous fat which has a low thermal conductivity will decrease the rate at which heat arrives at the surface and retard heat loss. The *epidermis* is a poor heat conductor. It is twice as bad as fat and three times as poor as the *corium*.

Every one knows that certain substances do not readily conduct heat. In the hot room of a Turkish bath, where all the objects are at the same temperature, metallic objects feel much hotter than those of wood, bone, rubber, etc. A familiar example of the retention of heat by a body surrounded by some bad conductor is found in the hay box or Norwegian cooker, which consists of a wooden box having a thick lining of felt. The partially cooked meal while still hot is placed in the box and the intervening space firmly packed with hay or paper. The felt-lined lid is closed and the meal left to cook itself. After several hours the temperature will have fallen only a few degrees. The Dewar or thermos flask depends on a vacuum as non-conductor.

Animals which have a good layer of subcutaneous fat lose heat much more slowly than lean ones.

Fat acts as a heat insulator and retards loss by conduction. On a moderately warm day, the obese person becomes uncomfortably warm. He is unable to eliminate heat with sufficient rapidity, and, as a consequence, his temperature rises, and may cause an increase in general metabolism amounting to 50 per cent. over that of a thin person. Aquatic mammals rely on their adipose tissue to protect them from a too rapid loss of heat.

(*d*) **Duration of exposure.** The loss of heat varies directly with the duration of exposure of the surface. In actual practice, this can be determined for non-living systems only, because, as we shall see, in the living body secondary reactions take place; alterations in the state of the surface and also of the deep-lying tissues are produced, rendering difficult the application of this law to living beings. If the student uses his common sense, however, he will readily see that, if all conditions are kept constant except time, *the amount of heat lost by conduction must vary directly with the time of exposure.*

(ii.) Temperature Gradient.

Newton's law states that the amount of heat lost by a body in unit time is proportional to the difference in its temperature from that of the surrounding medium. The warm body loses heat and becomes colder, while the environment becomes warmer. This loss and gain goes on till the body and its environment are at the same temperature. Inspired air or cold food is warmed to body temperature at the expense of body heat. The drinking of cold water may cause a temporary reduction of rectal temperature. Conversely, hot food and drink tend to increase the temperature of the body.

The heat lost by excretion depends not so much on this factor as on the amount and nature of the excreta. Compared with the total heat lost, the amount lost by the heating of inspired air, cold ingesta, and by the excreta is trivial.

(iii.) **Force of Wind.**

In still air the loss by conduction is very slight. Air is not a very good thermal conductor. A layer of warmed air soon forms an envelope round the cooling body and prevents rapid conduction. A very slight movement of the air may produce a very appreciable effect by driving off the warm particles and bringing cool ones into contact with the body. This may arise naturally by the formation of *convection* currents. The heated air expands and becomes lighter and so rises and allows the colder air to flow in.

The cooling effect of an air current is appreciable when the air moves at the rate of about 0·4 to 0·5 metre per second. A non-perceptible draught with a velocity of 0·2 metre per second playing on the naked arm may increase the heat loss over that experienced in still air by 19 to 75 per cent. depending on the temperature of the air. A moderate breeze at 8 metres per second (15 miles per hour) with a temperature of 20° C. causes more rapid chilling of a naked man than exposure to still air at 2° C. (see also p. 445).

(iv.) **Humidity of Atmosphere.**

Water is a better conductor of heat than is air. A moment's consideration of the feeling of cold in cold air before entering water at the same temperature and during the process will convince one of this. We have already mentioned the difference in the cold sensation caused by a dry cold wind compared with that produced by a wind of even a much higher temperature but laden with moisture.

III. Evaporation of Moisture

As the temperature of the air and of the body approach the same value, the heat lost by the body by radiation and conduction will be exactly balanced by the heat absorbed from the environment by the body. If the atmosphere attain a higher temperature than the body, then these means of heat elimination will become inadequate, and the body temperature would increase synchronously with that of its surroundings. That normally this is not so is common knowledge. Many interesting instances have been known of persons submitted to high temperatures, and an examination of some of these cases may lead us to a knowledge of the mechanism called into play to prevent untoward results. On one point, preliminary emphasis must be laid, viz. the times during which these extreme temperatures were endured were of very short duration.

TABLE LXIX

Time to Kill Spores of Hay Bacilli at Various Temperatures

Temperature.	Time.
100° C.	over 16 hours
105–110°	2–4 hours
115°	30–60 minutes
125–130°	5–6 minutes
135°	1–5 minutes
140°	about 1 minute

That the time factor is of immense importance may be adduced from the preceding experiment (Table LXIX.) by Christen. In warm-blooded animals, 45° C. is generally considered a lethal temperature, but death may occur at a lower temperature (42°), provided the time of exposure is sufficiently prolonged.

Bakers' assistants are known regularly to have entered ovens heated to over 126° C. When the temperature approached 160°, they experienced extreme superficial vasodilatation. Young girl labourers are said to experience no inconvenience from a stay of 5-10 minutes in a kiln heated to about 130° C. Chaubert, the "Fire King," is reported as having withstood a temperature of between 226° and 315·5° C. Yet immersion in a bath of water at 45° C. is unbearable.

It is a matter of common knowledge that those who habitually work in abnormally overheated places consume large quantities of fluid and sweat profusely. The latent heat of water is the greatest of all substances known. *Every gram of water which is vaporised entails the use of* 536 *calories.* This is in accordance with the principle of energetics laid down by Le Chatelier (p. 9 *et seq.*). In general, if any change is brought about by the incidence of energy, then alteration will take place in the substance acted on to nullify these changes, *i.e.* a reaction of opposite direction occurs.

Substances which expand on heating will be cooled by mechanical expansion. Water increases its volume in passing from the liquid to the gaseous state, and therefore, as the result of such an expansion, the parent fluid is cooled. The evaporation of moisture from the surface thus causes cooling of the body (see also clothes, p. 452).

The quantity of heat lost by the evaporation of moisture (*a*) in the bronchial passages, etc., (*b*) in the form of sensible and insensible perspiration, depends mainly on five factors, viz.:

1. Area and nature of moist surface exposed.
2. Colour of moist surface exposed.
3. Gradient of temperature between body and environment.
4. Force of wind (partial pressure equilibrium).
5. Humidity of the air.

(1) The effective area of surface exposed to cooling depends

in great measure on the state of the superficial blood vessels. Dilatation of these enormously increases the area. It is interesting to note that the water content of the tissues varies with the age of the person. Young and old people have a higher water content than adults in their prime (Chap. XXXV.). From age 20 to 50 man loses about 29 grams of water per hour in insensible perspiration. Boys and old men lose more.

(2) The effect of colour is merely a matter of the differential absorption of radiant energy producing local heating in proportion to the amount of energy absorbed and so causing a more or less rapid evaporation. The black *moist* muzzle of the bull-dog is much cooler than its white, comparatively dry skin.

(3) The gradient of temperature has much to do with the amount of heat lost by evaporation. This is an indirect effect. It has been suggested that the pigmented skin of tropical races, by absorbing radiant energy and so becoming unduly warm, stimulates epidermal nerve-endings and produces vasodilatation and profuse sweating. The evaporation of this water then cools the body in a similar way to the Indian *chatti* or water cooler. According to G. F. Hearne, exhaustion of the sweat mechanism always preceded heat-stroke in Mesopotamia.

(4) The presence of a current of dry air removes the gaseous water from the surface, and so brings dry air in contact with the body. In other words, the partial pressure of the gaseous water in the layer next to the body is kept at a minimum. This drying effect of wind is operative irrespective of the temperature of the wind, as witness the drying of shallow pools of water and of wet clothes by cold and by warm breezes.

(5) **Humidity of Air.** The presence of moisture in the air affects its cooling powers in two ways. (α) Obviously, if the air is already saturated with moisture it is unable to absorb more, and evaporation from the body even when played upon by a draught will be at a minimum. (β) On the other hand, as we have already mentioned, moist air is a much better conductor of heat than dry air, and, therefore, the heat lost *by conduction* tends towards a maximum in a humid atmosphere. The former of these factors plays a major part when the surroundings are warm (over 70° F.), the latter operates maximally in cold climates (under 65° F.). This effect of humidity is clear when one considers the conditions under which tobacco leaf is dried in Rhodesia. In the first stage (drying at 40° C.) it is impossible for any one to enter the drying sheds. The sheds are quite enterable during the second stage (at almost 100° C.). At this stage the leaf and, therefore, the air is quite dry, while at 40° the leaf is fresh and giving off moisture.

In the assessment of the conditions of the atmosphere making for ideal heat-loss, two instruments are in common use, viz., the wet and dry bulb thermometer and Hill's "Kata" thermometer (or its popular modification—the Comfimeter). The first of these instruments consists of two similar mercury thermometers fastened side by side on a stand. The reservoir of one is covered by a close fitting muslin bag which is kept moist by connection to a wick dipping in water. The water evaporates from the bulb at a rate depending principally on the degree of saturation of the atmosphere with aqueous vapour. It is obvious that, if the air is already saturated with moisture and no evaporation is taking place from the wet bulb, both thermometers will register the same temperature. On the other hand, if the difference in the temperatures recorded is great, one may consider that the air is dry.

Example. The degree of humidity of the air may be calculated from the formulæ,

$$\text{degree of humidity} = \frac{f}{m} \text{ and } f = F - AH(t - \theta),$$

where A = the constant of the instrument,
F = the tension corresponding to $\theta°$, the reading on the wet bulb thermometer,
H = atmospheric pressure,
t = reading on dry bulb thermometer,
and m = maximal vapour tension at $t°$ C.

If $A = 0{\cdot}00082$, $t = 20°$ C., $\theta = 16°$ C., $H = 758$ mm. Hg,
F (from a table of vapour tensions) $= 13{\cdot}63$ mm. Hg.

Then $f = 13{\cdot}63 - 0{\cdot}00082 \times 758 \times 4 = 11{\cdot}16$ mm. Hg.

The maximal vapour tension at 20° C. (from a table) $= 17{\cdot}52$ mm. Hg.

Therefore, the degree of humidity $= \dfrac{f}{m} = \dfrac{11{\cdot}16}{17{\cdot}52} = 0{\cdot}637.$

Valuable as this information is, it does not give us a true idea of the cooling power of any atmosphere. It fails to register the cooling due to air movement, for instance; so L. Hill devised the kata-thermometer, whereby the rate of cooling is measured directly.

The "Kata" thermometer is a large bulbed spirit thermometer with only two marks on the scale indicating a difference of 5° F. in their difference of level. That is, the top mark = 100° F. and the lower mark = 95° F., with a mean value about the mean value of body temperature = 97·5 F. or 36·5° C. The instrument has inscribed on its stem a factor F, relating principally to the cooling area of the bulb. The kata-thermometer is placed in warm water (about 105° F.) till the spirit rises and enters the top bulb. The bulb is now wiped dry and suspended well away from the body or any other source of heat. A stop-watch is started when the meniscus passes the top mark and stopped when it reaches the lower mark. Three to five readings are taken. The average time in seconds occupied by the meniscus in falling from 100° to 95° F. divided into F gives the cooling power of the atmosphere in that place due to conduction and radiation in millicalories per cm.^{2} per sec.

To determine the cooling power due to the evaporation of moisture a little silk-net finger-stall is fitted on the bulb and the experiment carried out as above. Excess water, of course, is removed from the finger-stall. Suppose $F = 470$, the dry reading = 78 seconds, and the wet reading = 26 seconds, then the loss of heat due to radiation, conduction and convection is just over 6 millicalories per square centimetre per second. The humidity of the

atmosphere raises this value three times. These values 6 and 18 are maximal values for the cooling power of the atmosphere in rooms where sedentary work is being carried out. For heavy physical work, the cooling power must be increased in proportion to the severity of the work.

It may be surrounded by wet muslin and the experiment repeated. The dry bulb instrument gives an indication of the possibilities of heat-loss by radiation and conduction, while the wet bulb indicates, *in addition*, the possibility of heat-loss by evaporation of moisture.

The Comfimeter is a form of kata-thermometer designed to give an immediate indication of the cooling property of the air in rooms. It consists of a cylindrical metal box in which is inserted an electric incandescent lamp.

On the top of the box is fixed an inverted metal funnel having a long stem. An ordinary thermometer is hung from inside the upper part of this stem so that about two-thirds of its length remains outside the instrument. When the lamp is lit, air enters the comfimeter by means of holes in the cylindrical box and the heated air rises and leaves by the orifice in which the thermometer is hung. The whole apparatus is cooled by radiation, convection and by conduction. When the comfimeter gives a reading of 30° C., this indicates a cooling power of about 7 millicalories per square centimetre of effective cooling surface per second—an ideal condition. If, now, the box be screened from draughts, the comfimeter thermometer will rise to 45° or maybe 50° C. On permitting free ventilation, the instrument will again record a temperature of about 30° C. A lower temperature than this is a sign of too rapid cooling. The designer, Professor L. Hill, says that as long as schools, factories, etc., are kept with the comfimeter indicating somewhere about 30° C., conditions suitable for work will be maintained.

Work of itself, as is well known, causes production of heat in the body. Some 75 per cent. of the energy generated in muscular contraction is dissipated as heat. In spite of the large quantity of heat thus liberated, the body temperature does not increase to any great extent. It may rise by about 1° C., depending on the cooling value of the environment as well as on the severity of the work. The following figures from Pembrey (Table LXX.) illustrate the influence of the rate of cooling on the body temperature after severe work :

TABLE LXX

SOLDIERS ON COMPLETING A MARCH OF SEVEN MILES

External Temp.		Increase of pulse rate.	Increase of temp.
Dry bulb.	Wet bulb.		
79	67·5° F.	62	1·4° F.
45	38° F.	14	0·8° F.

On the colder day with a percentage difference of about 15 between dry and wet bulb temperatures, *i.e.* under good cooling conditions, the temperature and heart-rate of the soldiers showed

only a slight increase, while on the warmer day with almost the same humidity, the men became uncomfortably hot, and the heart-rate was increased. Under these conditions man is not an efficient machine.

The effect of the temperature and the humidity of the external air on the loss of heat from the body is very well marked in those people endowed with a good layer of adipose tissue. As has already been mentioned, this dense non-conducting material is a very effective heat insulator, thus preventing rapid heat loss. In hot weather and during exercise, fat subjects become unduly heated. If, in addition, they have poorly functioning sweat glands, then severe or prolonged work becomes impossible.

The following table (LXXI.) gives a rough indication of the amount of heat lost per day through the various channels :

TABLE LXXI

	Per cent.	Calories per day.
1. Radiation and conduction	73	1,792
2. Evaporation (*a*) lungs, etc.	7·2	182
(*b*) skin	14·5	364
3. Excreta (*a*) CO_2	3·5	84
(*b*) urine and fæces	1·8	48
Total heat loss per day		2,470

The question now under consideration is, provided some 2,500 Calories of heat are lost per day, (*a*) how is excessive heat loss prevented, and (*b*) how is the amount lost made good ?

A. Physical Regulation—Preventative. (Usually effective up to 30° C. and over 40° C.)

This is obtained by decreasing the effective cooling surface, a result which may be produced in three ways :

1. By wearing clothes (or fur), a layer of still air is established between the body and the outer air. Heat will then be lost from the covered area by radiation only (see clothes).

2. A good layer of subcutaneous fat prevents a rapid diffusion of heat from the interior to the exterior of the body.

3. Constriction of the cutaneous blood vessels, a reflex act, results (*a*) in a decrease in the amount of blood carried to the surface, *i.e.* decrease in effective cooling surface, and (*b*) in a decreased output from the sweat glands.

Paralysis of the vasomotor nerves leads to vasodilatation. This is given as the reason for the death of animals whose skins

have been covered with a layer of impenetrable varnish. It is alleged that, at the enthronement of Pope Leo X., a boy was gilded to represent an angel. Before the ceremony was finished the boy, despite the efforts of Leonardo da Vinci and other physicians, had died. That excessive heat loss was the cause of this accident is proved by the experiments of Valentin. He showed that, under similar circumstances, the carbon-dioxide output of the subject was reduced to about one-sixth of the normal, *i.e.* metabolism was at a low ebb. If the animal were kept normally warm, the carbon-dioxide output returned to a normal figure. On the other hand, Senator states that the human body can be covered for 8 to 10 days with an impenetrable layer of varnish without producing any disturbance of metabolism. He avers that, in those cases where vasodilatation occurred, some toxic substance must be absorbed from the varnish.

Alcohol produces vasodilatation--an increased cooling surface. That is, this drug, because it causes more warm blood to come to the surface, gives rise to a *sensation* of cutaneous warmth while at the same time materially aiding in the depletion of the body's store of heat.

B. Chemical Regulation—Curative. (Usually predominant below 26° C. and over 40° C.)

These mechanisms come into play to *replace* heat which has been lost or when the surroundings are warm to prevent the body from liberating heat from potential energy.

(*a*) Voluntary or involuntary work. We have already seen that work causes the liberation of heat and we have discussed the reason for this. Shivering, or involuntary work is a reflex act which occurs in any person of normal muscular tone when the skin temperature is allowed to drop below a certain value. Anything which will prevent muscle from responding to this stimulus will do away with this curative heat production, *e.g.* curari, alcohol, etc. Young animals which have little muscular development are, obviously, unable to keep themselves warm by exercise and have to be carefully protected against undue heat loss.

(*b*) Increased muscular work ultimately leads to increased catabolism of food material and so, indirectly, to an increase in the quantity of heat liberated. Over and above this amount, however, the result of exposure to excessive cold may be combated by the ingestion of foods having a high energy value. Cold blood augments the sugar consumption of the heart-lung preparation, while warm blood has the opposite effect. Natives of the colder climates introduce much fatty matter into their diets, while

tropical dwellers cut down their protein intake (specific dynamic action).

(*c*) Subjection of an animal to great external heat leads not only to a disinclination to do external work or to take food, but definitely decreases the flow of the digestive juices. A dog with a gastric fistula (Pavlov, *q.v.*) gave neither a psychic nor a hormonic flow of gastric juice when its body was overheated.

One may just glance at a problem connected with the above. If the action of any tissue-complex, muscle, liver, etc., results in the liberation of "waste" heat, is heat then liberated during cerebral activity? At present, careful investigation has failed to show the production of heat by the brain. It is admittedly true that the head becomes heated during prolonged mental work, but this is accounted for by the increased cranial blood supply—blood flowing away from the extremities and from the viscera to the brain.

Centre. Since the means adopted for the maintenance of an unfluctuating temperature involve the bringing into play of so many structures and of such a variety of nerves—vasomotor, muscular, secretory and so on—it is plain that a co-ordinating centre is a necessity. Experimental evidence leads one to the conclusion that such a centre exists in the *corpus striatum*. Vaso-constriction, shivering, and a rise in rectal temperature result from the stimulation of this structure with a cold object. On the other hand, the application of a warm stimulating point leads to vasodilatation, muscular relaxation, and a fall in body temperature. Such results are generally interpreted as indications of the sensitiveness of the corpus striatum to alterations of the temperature of the blood flowing through its capillary system.

Ventilation. The purpose of ventilation is to provide such a change in the air of rooms, that the cooling power of the atmosphere of the rooms shall be suitable for the nature of the work being done. Some experiments by L. Hill make this perfectly clear, and show how erroneous were the older ideas concerning ventilation. A number of healthy young men were crowded into an airtight cabinet, provided with an electric fan, and with the necessary apparatus for sampling the air for analysis, etc. "After 44 minutes the dry-bulb thermometer stood at 87° F., the wet bulb at 83° F. The carbon-dioxide had risen to 5·26 per cent. The oxygen had fallen to 15·1 per cent. The discomfort felt was great; all were wet with sweat and the skin of all was flushed. The talking and laughing of the occupants had gradually become less and then ceased. On putting on the electric fan and whirling the air in the chamber the relief was immediate and very great,

and this in spite of the temperature of the chamber continuing to rise. On putting off the fans the discomfort returned. The occupants cried out for the fans" (Leonard Hill). Long before the discomfort had become extreme *the oxygen percentage became so low that matches would not burn.* The disinclination to smoke cigarettes was not noticed until some time after it was impossible to light them. In other experiments, Hill allowed the persons *in the cabinet* to breathe fresh air through tubes and mouthpieces piercing the walls of the chamber, or induced people outside the chamber whose bodies were adequately cooled to breathe the "vitiated" air from the cabinet. The former subjects received practically no benefit from their "fresh" air, but suffered just as they did in the experiment quoted above. The starting of the fan brought relief as before. In the latter experiment the "vitiated" air breathed by those whose bodies were in moving air had no effect. They did not experience discomfort nor had they any headaches nor other after effects popularly supposed to be due to breathing "polluted" air. Clearly, then, neither the lack of oxygen nor the presence of carbon-dioxide or other excreted substance has any influence on the "badness" of the atmosphere of a room. Discomfort is due to lack of cooling power, *i.e.* to stagnation and the loading up of the air with moisture.

Clothes. The question of the maintenance of the human body in comfort is so closely associated with the nature of clothing that a few physical facts bearing on the nature and value of artificial protective and decorative coverings are not out of place here. Liebig made clear one aspect of the function of clothing, saying, "*Our clothing is, in reference to the temperature of the body, merely*

TABLE LXXII (FROM RUBNER)

Substance.	Coefficient of Conductivity.
Air	53×10^{-6}
Brown human hair	76×10^{-6}
Grey human hair	74×10^{-6}
Feathers (eiderdown)	57×10^{-6}
Horse hair	57×10^{-6}
Cotton cloth (smooth)	81×10^{-6}
Woollens	68×10^{-6}
Silk	68×10^{-6}
Cashmere wool	68×10^{-6}
Cambric	81×10^{-6}
Flannel	72×10^{-6}
Flannelette	75×10^{-6}

an equivalent for a certain amount of food." In other words, if we were to do without the protection afforded by clothing we would have to make good the heat thus lost by eating more food.

The protective value of clothing mainly depends (1) inversely on the thermal conductivity of the material, (2) on its power of absorbing water, and (3) on the arrangement of the fibres of the material in the cloth. The conductivity of various materials is given in Tables LXVIII. and LXXII.

Coefficient of Protection. In order to calculate the coefficient of protection of clothing one must know the thickness of the material. Table LXXIII. compiled by Rubner gives further information regarding the density of the material and of the amount of air enmeshed in the structure. This layer of imprisoned air, as we have already mentioned, has a greater protective value than mere thickness of material.

TABLE LXXIII

Substance.	Thickness in mm.		Mean Density.	Percentage volume of air enmeshed.
	Total.	Of the Skin.		
Black cat fur	12·6	0·6	0·0429	96·7
Black lamb's wool (Astrakan)	13·0	0·3	0·0484	96·4
Rabbit skin	13·0	0·5	0·0304	97·7
Musk rat skin	14·0	0·6	0·0576	95·6
Otter skin	17·0	0·9	0·0638	95·1
White bear skin	21·5	0·9	0·0582	95·5
Beaver skin	22·0	0·8	0·0514	96·1
Skunk	26·0	0·5	0·0410	96·8
Sheepskin	40·0	0·7	0·0461	96·4
Cashmere wool	0·37	—	0·364	72·0
Cambric	0·29	—	0·179	86·7
Silk	0·25	—	0·329	74·7

A man entirely clothed in a garment of a total surface (s) of 19×10^3 sq. cm., of a thickness (t) of 75×10^{-2} cm. and having a difference of temperature (d) on the two sides of 10° C., loses heat (Q) as shown by the following formula:

$$Q = \frac{c \times s \times d}{t} \text{ calories per second,}$$

where c = coefficient of conductivity,

i.e. $$Q = \frac{c \times s \times d \times 3{,}600 \times 24}{t \times 1{,}000} \text{ Calories per day.}$$

If the garment were of wool, $c = 68 \times 10^{-6}$, we have

$$= \frac{68 \times 19 \times 36 \times 24 \times 10^{-6} \times 10^3 \times 10^2 \times 10}{75 \times 10^{-2} \times 10^3}$$

$= 1{,}488{\cdot}3$ Calories per day.

The coefficient of utility of clothing materials may be determined by covering with them a number of similar copper spheres filled with warm water and finding how long they take to cool through 10° C. If the time taken to cool 10° C. by an unclothed sphere with an external temperature of 12° C. be taken as θ, and the time necessary for the same sphere (clothed) to cool to the same extent be t, then $t/\theta = U$ (coefficient of utility).

TABLE LXXIV (From Bergonié)

Clothing.	Values of U.
Cycling vest (tight fitting)	1·1
Woollen shirt	1·5
Black leather waistcoat	1·6
Flannel shirt	1·75
Rough tweed coat	1·9
Weatherproof cloak	2·1
Woollen undervest	2·5
Heavy greatcoat	4·5

The efficiency of clothing depends in great measure on the arrangement of the fibres of the cloth so as to enmesh air and thus form a stationary layer between the body surface and the outer air. The following table (Table LXXV.) from Lefèvre gives an idea of the protective value of clothing. His subjects were placed in a rectangular box through which a measured amount of air was passed. The temperature of the air just before it reached the body and immediately after leaving the body was taken. If M, the mass of air passing in t minutes, is heated by $\theta°$, then the heat lost per minute $= \frac{0{\cdot}237 \times \theta \times M}{t}$ Calories, where 0·237 is the specific heat of air.

It will be noticed that heat production increases with the lowering of the temperature of the wind and also with increasing the speed of the wind. It is obvious that, under similar conditions, the heat lost (and the heat produced) by the clothed is less than that of the naked subject.

The colour of clothes has also something to do with their pro-

TABLE LXXV

Rate of Wind. Metres per sec.	Temperature of air entering.	Loss per hour in Calories.		Ratios.	
		Naked.	Clothed.	Fast/slow.	Naked/clothed.
1·2	9° C.	134	98	$\frac{201}{134} = 1{\cdot}5$	$\frac{134}{98} = 1{\cdot}36$
3·8	9° C.	201	130	$\frac{130}{98} = 1{\cdot}32$	$\frac{201}{130} = 1{\cdot}45$
1·5	5° C.	185	143	$\frac{277{\cdot}5}{185} = 1{\cdot}5$	$\frac{185}{148} = 1{\cdot}3$
3·8	5° C.	277·5	172	—	$\frac{277{\cdot}5}{172} = 1{\cdot}61$
4·0	4° C.	313	170	$\frac{172}{143} = 1{\cdot}2$	$\frac{313}{170} = 1{\cdot}84$

tective action. Quite apart from psychological effects, certain colours conduce to warmth and others to coolness. This is a problem that exercised the minds of those responsible for the health of white troops in tropical countries. The final result of one series of investigations was to recommend the wearing of white clothes with a black lining. The student may find it an interesting exercise to furnish a reason for this.

Further Reading

L. Hill. "Sunshine and Open Air." Arnold.

CHAPTER XXXIII

TROPISMS—THE SLAVES OF THE LAMP

> "Behold the child, by Nature's kindly law,
> Pleased with a rattle, tickled with a straw:
> Some livelier plaything gives his youth delight,
> A little louder but as empty quite;
> Scarfs, garters, gold, amuse his riper stage,
> And beads and prayer-books are the toys of age,
> Pleas'd with this bauble still, as that before,
> Till tir'd he sleeps, and life's poor play is o'er." POPE.

IT may be laid down as axiomatic that once an object has come into equilibrium with its environment, it will remain so until some change in the environment disturbs the harmony. In other words, matter has inertia—moves when it is moved, stops when it is stopped, and alters only in so far as it is altered. All inorganic substances are not in equilibrium with their environment. Radioactive minerals, as we have seen, are characterised by undergoing considerable change—seemingly independent of the nature of their surroundings. It is a moot point whether living things may be brought under this general statement. That they have inertia both in the ordinary physical sense and functionally is undoubted. Moreover, that alterations in the distribution of energy in the environment do lead to apparently corresponding alterations in the organism will be granted by most workers in this field, but all are not agreed as to how far the interpretation can be applied to animals high in the scale.

I. One of the best investigated phenomena in this line of study is that of **heliotropism** or phototaxis. It is well known that radiant energy is capable of influencing the rate of some chemical reactions—in proportion to the intensity of the light. This is known as the **Bunsen-Roscoe Law**, which may be formulated as:

$$it = \text{constant},$$

where i is the intensity of the light and t the time of exposure.

(1) Certain animals and free-swimming plant-organisms move towards or away from the source of light. These are said to be positively or negatively heliotropic respectively. Caterpillars of *Porthesia chrysorrhoea* are of the former class. They move towards the light and may starve, with abundance of food just behind them.

(2) If positively and negatively heliotropic animals are placed

in a trough covered half with red and half with blue glass, those that are positively heliotropic collect at the blue end and the others at the red end of the trough. Red glass is practically opaque, as every photographer knows, to the photo-chemical rays of light. The most efficient rays for heliotropic reactions are (*a*) the blue between 460 and 490$\mu\mu$ and (*b*) the yellow-green between 520 and 530$\mu\mu$. Now, most blue glass permits not only the passage of the blue rays, but of the yellow-green rays also (cf. Fig. 1).

(3) That the heliotropic animal is orientated in relation to the *source* of light is shown by a simple experiment due to Loeb. Direct sunlight is allowed to fall from the upper half of a window on to a table and diffused daylight from the lower half on to the same table on which is placed a test-tube in such a way that it lies at right angles to the window, and is illuminated over one-half of its length (room half) by direct sunlight and over the remainder by diffused daylight. Positively heliotropic animals are introduced into the sunny end of the tube. They promptly and invariably move towards the window, *i.e.* out of the sunlight into the shade *towards the source of light.*

(4) To explain these facts (and others), Loeb has put forward an interesting theory. "Animals possess photosensitive elements on the surface of their bodies, in the eyes or occasionally also in the epithelial cells of their skin. These photosensitive elements are arranged symmetrically in the body, and through nerves are connected with symmetrical groups of muscles. The light causes photochemical changes in the eyes (or photosensitive elements of the skin). The mass of photochemical reaction products" so formed "influences the central nervous system and through this the tension or energy production of the muscles. If the rate of photochemical reaction is equal in both eyes, this effect on the symmetrical muscles is equal and the muscles on both sides of the body work with equal energy; as a consequence the animal will not be deviated from the direction in which it is moving. This happens when the axis or plane of symmetry of the animal goes through the source of light, provided only one source of light be present. If, however, the light falls sideways on the animal the rate of photochemical reaction will be unequal in both eyes and the rate at which the symmetrical muscles on both sides of the body work will no longer be equal; as a consequence, the direction in which the animal moves will change. This change will take place in one of two ways, according as the animal is either positively or negatively heliotropic."

In mammals, at least, the rods of the retina (*q.v.*) appear to be

the elements sensitive to light. Rodless mice are not phototropically sensitive (Keiler).

(5) If this is true, it follows that the animal will obey the Bunsen-Roscoe Law. This is rather troublesome to prove for free-moving animals. The following table shows the applicability of the law to regenerating polyps of *Eudendrium*. The intensity of the light was altered by varying the distance between the source of light and the polyps.

TABLE LXXVI

Distance between Polyps and source of light (metres).	Time in minutes required to cause 50 per cent. of Polyps to bend towards the source of light.	
	Observed.	Calculated.
0·25	10	—
0·50	35–40	40
1·00	150	160
1·50	360–420	360

The calculated values of t tend to be somewhat larger than the observed results. Schwarzschild observed that when development followed exposure to light the formula should be modified to

$$it^p = \text{constant}.$$

For silver bromide gelatine plates, the value of the exponent p varies between 0·8 and 1 according to the brand of the plate.

Talbot's Law is the Bunsen-Roscoe Law modified to make it applicable to intermittent light. Intermittent light is as effective as constant light of the same intensity provided that the total duration of the intermittent light is equal to that of the constant light.

(6) What is going to be the result when the organism is subjected to light from two sources? One might predict that, if Loeb's hypothesis is correct, the organism will be orientated so that it comes to rest in a position where it is symmetrically stimulated. (*a*) If the two sources of light are of equal intensity and duration and are set at an equal distance from the organism it should be orientated with its plane of symmetry at right angles to the line joining the sources of light. (*b*) If the lights are of unequal intensity, the animal should move so that its photosensitive elements are in a position to absorb equal amounts of light energy. Further, the ***absolute intensities*** of light should have no effect on the deviation of the path of the organism from the straight path

outlined at first. *The relative intensities should be the governing factor.* These three predictions have been amply proved experimentally. The following results (Table LXXVII.) from Patten's investigations illustrate the nature of the findings.

TABLE LXXVII

No. of lamps used.	Difference of intensity between the two lights in percentages.		
	0	25	50
	Deflection in degrees.		
1	0·55	9·04	19·46
2	0·1	8·55	22·28
3	0·45	8·73	20·52
4	0·025	9·66	19·88
5	0·225	8·30	19·28

This table shows clearly that (col. 1) when the intensity of the two lights was equal, the animal varied on the average only 0·09 degree from the line of the original path. It also demonstrates that when the one light was reduced to three-quarters (col. 2) the intensity of the other, the angle of deviation was about 8·86, and that when a further reduction to a half was made, the angle of deviation was more than doubled. Finally, the figures show that *the angle of deviation depends on the relative differences of light intensity and is independent of absolute intensity* (provided sufficient light is present to overcome inertia) (cf. stimulation, p. 235).

(7) A model with a heliotropic mechanism has been constructed by Hays Hammond, the inventor of the dirigible torpedo. The principle on which the machine depends is the alteration in the electrical resistance of metallic selenium when exposed to light. The "eyes" are lenses separated from each other by a projecting "nose" which permits the shading of one eye while the other is illuminated. The lenses are each focused on separate selenium cells. The heliotropic machine consists of a rectangular box about 3 ft. long, 1½ ft. wide and 1 ft. high mounted on three wheels, two of which are geared to a driving motor. The third wheel, mounted at the rear-end, controls the steering. It may be turned right or left by the differential action of two solenoid electromagnets. The selenium cells are in circuit both with the driving motors and with the steering magnets. In the former case, the selenium cells control a series of very sensitive relays (cf. nervous system) in such a way that the amount of energy sent through the

driving motors and hence their speed is determined by the intensity of the light falling on the lenses. The steering magnets are opposed, *i.e.* if both selenium cells are illuminated equally, both magnets will receive the same current and the steering wheel will lie parallel to the driving wheels. If more light falls on one selenium " retina " than on the other, the former has its power to conduct electricity increased in proportion to the relative increase in intensity; consequently the magnets controlling the position of the rear wheel are activated asymmetrically. The wheel is pulled over to make an angle with the previous line of traction of the " dog." The mechanism is so arranged that this steering movement turns the machine towards the light. It will continue to turn till both lenses are equally illuminated. As soon and as long as both " eyes " are equally illuminated in sufficient intensity, the machine moves in a straight line towards the source of the light. The apparatus is fitted with a reversing switch which will convert it from a positively to a negatively heliotropic machine.

If, say, a portable electric light be turned on in front of the machine it will immediately start to follow (or run from) the light at a speed which may attain to 100 yds. per minute. On reducing the intensity of the light, the " dog " will slow down, and on switching off, it will stop. In this way the machine follows a lantern in a dark room just like a positively heliotropic animal. By reversing the direction of the current one may make the machine negatively heliotropic.

II. **Geotropism.** Most animals so orientate themselves that their plane of symmetry passes through the centre of the earth. They, therefore, either move towards the earth and are positively geotropic like the tap roots of plants or, more commonly, are negatively geotropic and climb. Rodless mice or other blind animals if placed in any position on a steeply inclined plane, after a few orientating movements, move straight up the plane. The steeper the plane, the fewer are the initial exploratory movements.

III. **Stereotropism** is the term applied to the tendency of certain organisms to bring their bodies as much as possible on all sides in contact with solid bodies. " The butterfly *Amphipyra*, which is a fast runner, will come to rest under a glass plate when the plate is put high enough above the ground so that it touches the back of the butterfly." Man orientates himself partly by apprecia-tion of the tactile influences on the soles of the feet. When these are weakened as in locomotor ataxia, and when the orientating influence of the eyes is removed, the patient finds difficulty in standing and in walking (Romberg's sign).

IV. **Chemotropism** plays an important part in the life of the

lower organisms. By it, the animal is drawn towards or draws away from certain chemical substances. The organ stimulated asymmetrically is orientated so that the stimulating impacts on it are symmetrical (see Chap. XII.).

V. **Galvanotropism.** It is easy to see that a simple animalcule, like amœba, would come readily under any influence which altered either its surface energy or the distribution of its colloidal contents or both. We have seen how colloids are attracted according to Hardy's Rule (*q.v.*) to anode or cathode according to which side of their isoelectric point the *p*H of the medium lies.

The isoelectric points of all body proteins (Table XIV.) are on the acid side of *p*H 7, and, therefore, if free to move, they would tend to collect on the side of the cell nearest the anode (p. 92). Either this produces some changes in the tension of the cell (cf. geotropism) or the lipoids or some other factors unknown as yet come into play and produce movement towards the cathode. Various experiments indicate that the former hypothesis is, at least, plausible.

VI. **Orientation in space** is determined mainly by three factors, light, tactile sense and gravitation. Normal equilibrium or normal geotropic orientation is defined as that position in which the plane of symmetry of the animal passes through the centre of the earth. Any deviation from that position causes unilateral stimulation and corrective movements are instituted. The tight-rope walker perceives that his centre of gravity is tending towards unstable equilibrium and, voluntarily (though generally subconsciously), corrects his balance. In the labyrinths, we have a delicate mechanism for detecting alterations in our orientation in space (*q.v.*).

Crozier and his co-workers have applied simultaneously two types of stimuli to which their experimental animals were sensitive. For example, negatively phototropic animals which when left in the dark would follow a path perpendicularly straight up a vertical plate (negatively geotropic) were subjected to a series of rays of light applied at right angles to and in the same plane as their path. The result invariably was a deviation of the path through an angle which was constant for that type of animal for each intensity of light. One can then standardise the geotropic intensity as equal to the effective light intensity when the angle of deviation is 45 degrees from the perpendicular.

In the same way one could apply any two other types of stimulation, and so on till all types of stimulation were standardised as to their tropic intensities in terms of one another. One would then be able to predict the angle of deviation of the path of any tropic

animal under any circumstances. Take, for example, the triclad *Leptoplana variabilis*, which is cathodally galvanotropic and negatively phototropic, and place it in a black rubber photographic developing dish. The dish is furnished with cotton electrodes at either short end and a potential difference of about 0·4 volt established between them. Three opal electric lamps placed at one side of the dish supply the light. When the light is off, the planaria move straight to the negative electrode, and, when the current is off, they move straight away from the source of light. By varying the intensity of either form of stimulation, it has been found that the triclad moves half-way between these extreme paths, *i.e.* with an angle of deviation of 45° C. when the current intensity is proportional to the logarithm of the intensity of the light (Crozier and Steer) (see also Adaptation, Chap. XXXIV.).

Sufficient has been said to show the nature and indicate the mechanism of those actions termed tropisms. In principle they depend on unilateral stimulation of a symmetrical animal. How far they can be accepted as explanations of all the instinctive actions of the lower organisms or of any of the actions of the higher animals remains an open and debatable question.

CHAPTER XXXIV

ADAPTATION

"The free use of final causes to explain what seems obscure was temptingly easy. . . . Hence the finalist was often the man who made a liberal use of the *ignava ratio*, or lazy argument: when you failed to explain a thing by the ordinary process of causality, you could 'explain' it by reference to some purpose of nature or of its Creator."

PRINCIPAL GALLOWAY quoted by D'ARCY THOMPSON.

If the environment exerts such an all-powerful effect on the organism, can the organism alter itself according to the principle of Le Chatelier so that it may live with the least possible expenditure of energy? That is, has the animal the power of adaptation? There is no doubt whatever as to the adaptation of growing bone or growing tissue of any sort to the stresses and strains incident upon it. Various organs are known to adapt themselves to meet alterations in the conditions under which they work.

When one comes to consider the organism as a whole, the evidence for adaptation is not so conclusive. The Arctic fox and the polar bear are not white because they have adapted themselves to a white background, but because their coloured relatives have paid the penalty consequent on their easy visibility against a white background. It has been said that trypanosomes may be obtained which are almost unaffected by treatment with arsenic. The process for producing them is to give their host a high but non-lethal dose of arsenic, infect another host with the survivors and so on. This is clearly a case of the survival and propagation of the most resistant strains.

Animals which live in dark or semi-dark places have generally defective eyesight. Is this due to atrophy from want of use or might one not argue that the environment of the cave was the fittest for the blind or semi-blind animal? Not only would they be at a manifest disadvantage in the struggle for existence outside, but they have a distinct advantage in the cave over any seeing animal that may stray in.

To be brief, one must consider that, as anything but a rapid response to the distribution of forces in the environment is incompatible with life, the animal capable of adapting itself to circumstances will live and probably propagate. Man, because of

his highly organised nervous and muscular systems, is able to adapt himself readily and, therefore, reigns supreme.

In the previous chapter we referred to some experiments in which planarians were subjected to two stimuli—geotropic and phototropic—and stated that the movements of the animal were governed by the angle of incidence and relative intensities of the effective stimuli falling on it. Crozier and Wolf found on carrying out repeated experiments on the same animals that adaptation to light gradually took place. This adaptation occurs according to a definite mathematical formula, so that one could predict, for any intensity of light, how soon the animal would cease to be influenced by it and follow undisturbed its geotropic path. Some change had, therefore, occurred in the protoplasm of the animalcule which rendered it insensitive to light.

A somewhat different type of experiment leads us to the same conclusion. If we arrange the stage of a microscope so that a tiny strongly illuminated square appears in the field, and observe the movements of an amœba near the square, we will find that, by chance, a pseudopod will enter the illuminated part for about 1 or 2 microns. Protoplasmic flow in the pseudopod will stop for a moment, then begin again, but in the reverse direction. Finally, the pseudopod will withdraw from the illuminated area. Later, another pseudopod will be advanced towards the square, thrust in, and the above process repeated, and so on. "After the animalcule had repeated this process several times, thrusting one pseudopod after another into the square, a general change in the course of action took place. Pseudopods were no longer formed on that side of the organism. It was noted that the number of attempts to enter the square decreased with every succeeding pseudopod formed, till with the last pseudopod thrust out in that direction a single attempt sufficed and the square was barely entered. Repeated experiments with the same specimen of *amœba proteus* led to such a change in the protoplasm that after ten or fifteen experiments the moment the organism touched the square it withdrew that pseudopod and, thrusting one out in the opposite direction, *moved away from the light*" (Mast).

McClendon, in a modification of Mast's experiment, tapped the amœba with a glass rod, and found that the strength of the stimulus, the number of shocks and their frequency, all influence the response. That is, the amœba can profit by experience and be taught just like the higher animals.

Careful examination of the protoplasm during these lessons shows that a different mass of protoplasm is thrust into the square each time in any one experiment. That is, the shock leaves some

record on the protoplasm. This record or " engram " is the basis of memory, and the ability of protoplasm to retain the engraphic record, *i.e.* its teachableness, is the basis of adaptation. " The engram has a nature which is essentially dynamic. It is not to be thought of as a mote left in the protoplasm by the stimulating agent. It is rather a process." (Bovie.)

No case is known where acquired characters have been transmitted to offspring.

On the other hand, the environment may have profound effects, not in the nature of adaptation, but on the development of the organism.

Temperature. In Chap. XXXII. the effect of alterations in temperature on physical, chemical and physiological phenomena was considered. Temperature influences all life phenomena.

(*a*) **Development.** One of the simplest experiments of this nature is to determine the temperature coefficient of the development of an egg. Usually the egg of the sea-urchin is chosen for this purpose. Table LXXVIII. (Loeb and Chamberlain) gives the time in minutes required from insemination to the first cell-division for various temperatures.

TABLE LXXVIII

Effect of Increase of Temperature on Cell-division in Egg of Sea-urchin

Temperature (° C.) .	8	10	15	20	25
Time (minutes) . .	411	208	100	56	39·5

Increase of temperature thus causes a more rapid development of the egg.

(*b*) **Rate of Metabolism.** Increase of temperature, within limits, as we have seen, causes an increase in general metabolism. More oxygen is used, more carbon-dioxide is excreted, etc. Organs work at a greater rate, *e.g.* the heart beats more rapidly. The alterations of the rate of the heart of *Fundulus* (embryo) keeps such regular pace with alterations in external temperature that it could form the basis for a rough thermometer, as Table LXXIX. shows. From the figures we are also justified in inferring that the influence of temperature in this reaction is a function of this particular temperature and does not depend on whether the organism is gaining or losing heat.

(*c*) The time necessary to reach sexual maturity is decreased by increase of temperature. Stefansson reports that the Eskimo

TABLE LXXIX

RELATION BETWEEN TEMPERATURE AND RATE OF HEART BEAT IN FUNDULUS EMBRYO

Temperature (° C.) . .	20	15	10	5	10	15	20
Time for 19 beats (minutes) .	11·5	19·0	32·5	61	33·5	18·8	12

girl usually has offspring by her twelfth year. This early maturity, he states, may be attributed to the fact that the Eskimo keeps his body at a temperature as high if not higher than that of dwellers in Southern Europe. Be that as it may, other observers have noticed that growth in height and in weight is increased during periods of increased temperature, *e.g.* summer (see Growth).

CHAPTER XXXV

GROWTH

"The living organism is so constituted that each disturbing influence stimulates it to put in action a compensatory mechanism which will neutralise and render innocuous the disturbing agency." FRÉDÉRICQ.

GROWTH may be considered as an attempt of a system to get into equilibrium with its environment. Generally, but not invariably, increase in size is accompanied by changes in external form and in internal structure. Development is, in most cases, a necessary result of growth. This chapter will deal with increase in size quite apart from any concomitant alterations in complexity.

I. Nature of the Phenomenon.

At first sight it seems easy to distinguish between a mere accretion of material like crystal growth, snowball increase or accumulation of interest on capital, and organic growth. A more careful examination of the cause and resultant velocity of growth shows that in both inorganic and organic worlds similar principles are involved, and that similar factors operate towards similar ends.

A series of interesting and illuminating experiments emanating from Leduc's laboratory are suggestive. If a little seed compounded of copper sulphate and glucose be planted in a gelatine (1 to 5 per cent.) gel, through which a little potassium ferrocyanide has been dispersed, growth will take place. In the first place, by the interaction of copper- and ferrocyanide-ions, a membrane of copper ferrocyanide will be formed round the seed. This membrane is semi-permeable, allowing free passage of water but preventing the egress of the crystalloid ions. As a result, the seed, thus gaining water by endosmosis, will swell up and, when the elastic limit of the membrane has been reached, will burst. Immediately this happens, a new membrane will form round the copper-glucose solution and the process will be repeated. By this means remarkable life-like growths are obtained. (Details of preparation are given in Part II., p. 544).

Botanists are agreed that osmosis plays an important part in plant growth. An experiment is given in Part II., p. 515, to illustrate the production of turgescence and consequent rigidity

as the result of endosmosis (Fig. 45). Plant growth is conspicuously associated with *turgor*, and depends in great measure on the amount of water taken up. Another and more plausible explanation may be given of the swelling of plant tissues. In Chap. VIII., p. 97, Table XVII., we mentioned the power of colloids to imbibe and compress water. It is extremely probable that plant turgor may be due to this imbibition, initiated by some alteration in the hydrogen ion concentration of the tissue.

It has been definitely proved that animal growth is accompanied by alteration in water content as shown in Tables LXXX. and LXXXI.

TABLE LXXX

WATER CONTENT OF HUMAN EMBRYO (FEHLING)

Age (weeks).	Weight (grams).	Increment.	Increment per week.	Water per cent.
6	0·975	—	—	97·5
17	36·5	35·525	3·23	91·8
22	100·0	63·5	12·7	92·0
24	242·0	142·0	71·0	89·9
26	569·0	327·0	163·5	86·4
30	942·0	355·0	88·75	83·7
39	1640·0	716·0	79·56	74·2

WATER CONTENT OF FROG EMBRYO. (DAVENPORT.)

Age (weeks) .	1	2	5	7	9	14	41	84
Water per cent. .	56·3	58·5	76·7	89·3	93·1	95·0	90·2	87·5

TABLE LXXXI

PERCENTAGES OF FREE AND BOUND WATER IN ANIMAL TISSUES AT CERTAIN AGES (FROM THOENES)

Animal.	Age.	Total Water per cent.	Free Water per cent.	Bound Water per cent.	Bound Water (grams) for each gram of Dry Matter.
Guinea pig .	Young (160 gm.) .	81·6	61·5	20·1	1·09
,, .	Old (600 gm.) . .	79·6	60·5	19·2	0·94
Dog .	1 day . . .	85·7	59·0	26·7	1·86
,, .	3 weeks . . .	83·8	60·4	23·4	1·44
,, .	4 weeks . . .	83·3	59·7	23·6	1·40
,, .	Several months . .	79·3	55·1	24·2	1·16
,, .	Several months . .	82·0	62·9	19·1	1·06
,, .	Several months . .	79·7	58·3	21·4	1·05

Free and Bound Water. Reference to the table above (LXXXI.) will make it clear that this change of water content with age refers almost exclusively to the bound water (*q.v.*) which we saw in an earlier chapter is held with extreme avidity by the tissues.

In the later stages of growth and especially in the higher mammals the ratio of water to solids tends to diminish. Inhibition of growth occurs when means are taken to prevent the entrance of water. For example, Loeb put *Tubularia* and *Cerianthus*, which live and grow in sea-water having about 3 to 3·5 per cent. salts, into a more concentrated mixture. He found when the concentration of salts in the water was 5·4 per cent. that these organisms ceased to grow. The water-holding power of the salt solution, *i.e.* the exosmotic property of the artificial sea-water, balanced the inwards pull of the protoplasm.

II. Normal Rate of Growth.

(*a*) **Weight.** Brailsford Robertson has shown that the rate of increase in weight follows a curve characteristic of *autocatalytic* reactions, *i.e.* of reactions in which one of the resultant products acts as a catalyst for the whole reaction. A simple example of a reaction of this type may be found in the inversion of an aqueous solution of cane sugar at 100° C. Part of the product of the reaction (glucose and fructose) appears to undergo further decomposition, giving rise to an unknown acid which accelerates the rate of inversion.

If x denote the amount of invert sugar formed during hydrolysis, x will also be proportional to the amount of acid produced. Now, by the ordinary *compound interest law* in which a function varies at a rate proportional to itself—an exponential function—we have :

$$\frac{\delta x}{\delta t} = kx(a - x),$$

or, on integrating,

$$k = \frac{1}{at} \log \frac{ax}{a - x},$$

where k is a constant.

As the result of a series of experiments on auto-inversion at 100° C., the value of k for this reaction has been put $= 122 \times 10^{-6}$. With this value we can tell at any time after the inception of the reaction just how much sugar has been inverted. Further, it is obvious that, as the action proceeds, the velocity due to the concentration of the original substance gradually decreases (*i.e.* the ordinary mass action without the catalyst), while that due to the concentration of the newly formed substance keeps steadily increasing. Hence, there must, at a certain time, be a maximum

velocity due to definite concentrations of a and x. In an auto-catalytic reaction this maximum velocity is reached when the concentration of the newly formed substance is half the concentration of the original substance, *i.e.* when $x = a/_2$.

Do statistical results bear out the statement that growth is an autocatalytic reaction? In the following table (LXXXII.) is given for comparison, the weight of the human body at various ages, as found and as calculated from the assumption that the rate of growth is autocatalytic.

TABLE LXXXII

Age (years).	Body weight (Kg.).		Number of cases.
	Found.	Calculated.	
0·5	3·4	3·4	100
5·5	22·7	16·5	176
6·5	24·6	20·1	327
7·5	25·9	23·4	631
8·5	27·0	26·2	1,038
9·5	28·3	28·4	1,262
10·5	30·4	30·4	1,200
11·5	32·3	32·2	1,129
12·5	35·0	34·0	863
15·5	47·1	44·8	1,451
20·5	66·1	65·4	459

From these results we see that the agreement between observed and calculated results is excellent in all cases where a sufficiently large number of subjects have been weighed, except at age 15½, where weight increases more rapidly than theory demands.

A simple but empirical formula for obtaining "*expected*" *weight* is $\frac{A}{4{\cdot}75m}$, where A = conceptional age in years and m is a constant for each age (see Table LXXXIII. for values of m and for results).

(*b*) Length. It was at first believed that the length curve was of parabolic form of the equation $y^2 = a(x + b)$, but later and more complete investigation has shown that this is untenable. There is for each type of body a definite relationship between length and weight, viz.: $l = \sqrt[3]{mw}$, where l = length in metres, w = weight in kilograms, and m is a constant for each age (and each type). As $w = \frac{A}{4{\cdot}75m}$, therefore $A = 4{\cdot}75l^3$ or $l = \sqrt[3]{\frac{A}{4{\cdot}75}}$. (Pfaundler.)

Pfaundler gives the following examples:

(i.) A boy aged 1 year has a conceptional age $A = 1{\cdot}75$ yr.

Hence length $= \sqrt[3]{\dfrac{1{\cdot}75}{4{\cdot}75}} = \sqrt{0{\cdot}36} = 0{\cdot}72$ metre, which compares favourably with the value given in Table LXXXIII.

(ii.) A boy aged 8 yrs. has $A = 8{\cdot}75$.

Hence length $= \sqrt[3]{\dfrac{8{\cdot}75}{4{\cdot}75}} = \sqrt{1{\cdot}74} = 1{\cdot}23$ metres.

(Average length of boy of 8 yrs. $= 1{\cdot}20$ metres.)

TABLE LXXXIII

Age from birth.	Conceptional age A (yrs.)	Length (metres).		Constant m.	Weight (Kg.).		$\frac{\text{Weight}}{\text{Height}} \times 100$.
		Found.	Calc.		Found.	Calc.	
3 months (fœtal)	0·225	0·08	—	25·6	20×10^{-3}	—	0·025
6 ,, (fœtal)	0·450	0·31	0·45	46·91	635×10^{-3}	—	2
8 ,, (fœtal)	0·600	0·42	0·50	36·1	$2{,}100 \times 10^{-3}$	—	5
10 ,, (fœtal)	0·750	0·50	0·53	39·73	$3{,}300 \times 10^{-3}$	$3{,}975 \times 10^{-3}$	6
1 month	0·833	0·54	0·55	38·72	4·25	4·53	8
2 months	0·916	0·58	0·58	40·24	4·95	4·79	8·5
3 ,,	1·008	0·61	0·60	41·33	5·6	5·09	9
6 ,,	1·25	0·66	0·64	40·56	7·2	6·50	10
9 ,,	1·50	0·70	0·69	40·74	8·6	7·75	12
1 year	1·75	0·74	0·72	42·88	9·4	8·59	12·6
2 years	2·75	0·84	0·83	48·98	12·1	11·82	14
3 ,,	3·75	0·90	0·92	55·06	13·2	14·34	14·6
6 ,,	6·75	1·11	1·12	68·17	18·1	17·76	16·3
9 ,,	9·75	1·24	1·27	78·41	24·9	26·15	20
12 ,,	12·75	1·38	1·39	84·94	30·9	31·60	22

Table LXXXIII. is from Pfaundler's data. It gives the calculated and observed weights for each age as well as his values for m and the centimetre index.

The ratio, weight in Kg./length in centimetres, is called the **centimetre index.** Sometimes the ratio is modified as in Levi's ratio, which is

$$\frac{100\sqrt[3]{\text{wt. in grams}}}{\text{length in cm.}}.$$

This ratio diminishes as age increases up to puberty.

Dreyer suggests that instead of the total length of the body it would be better to deal with the *sitting height.*

He has established the following ratios:

Males, $W = 0{\cdot}38025\sqrt[0{\cdot}319]{l}$;

Females $W = 0{\cdot}36093\sqrt[0{\cdot}313]{l}$;

where W = weight of the body in grams.

and l = length of trunk in cm.

Note.—The values of m in Table LXXXIII. have been multiplied by 10^3 for convenience.

Some organisms when their size reaches a certain limiting value tend to divide into two equal portions (Sach's Rule). Therefore, one has to deal with an increase in number quite apart from increase in individual size or weight. It has been proved by numerous experiments that the increase in the number of cells follows the *compound interest law*, *i.e.* is an autocatalytic reaction (*q.v.*).

To summarise : When it is said that growth is an autocatalytic reaction it is inferred that (i.) superimposed on the velocity of reaction, which may be classed as chemical and is governed by the law of mass action, (ii.) there is a variation in rate due to the presence of a catalyst in one of the products of the main action. The phases of such a reaction are, at least, four :

(1) Ordinary velocity, proportional to the mass of the reacting bodies.

(2) After a short period the catalyst makes its appearance, and the total rate gradually and steadily increases.

(3) Certain limiting factors probably caused by the presence in the blood (or in the sap of plants) of endocrinetes inhibit too rapid a growth.

(4) The accumulation of the products of the reaction produces a tendency to cause a reaction in the reverse direction. That is, arrest of growth and even *negative growth* may be produced.

Quetelet, who was the pioneer of the statistical study of growth, found that the rate of growth alters with age in a definite orderly way; and the velocity curve may be divided into fairly well-defined regions, each having a definite and characteristic slope, *e.g.*

(1) From conception to about 3 lunar months the velocity is low, about 2 cm. per month.

(2) Period of rapid growth from 3 to 9 lunar months = 9 cm. per month.

(3) Rate almost equal to (1), *i.e.* 2 cm. per month from birth to 3 years or so.

(4) Slower, but still rapid growth in early boyhood. (Marked quickening in teens (growing age).)

(5) Period of arrest—full stature has been reached.

(6) Somewhere about 50 years of age the period of *negative growth* sets in. *That is, the curve of growth and picture of velocity follows point by point the velocity curve of a typical autocatalytic reaction.*

III. Factors Modifying Growth.

Chemical reactions may be profoundly altered by alterations in external conditions, and, therefore, we may expect to find

certain variations in the rate of growth which may be correlated with alterations in the conditions to which the subjects are subjected.

1. Phase Differences.

(*a*) **Individual.** Quetelet found that, under normal conditions, the *variations* in the rate of growth of man were just what might be predicted from the application of the mathematical law of probability. This law is represented by the equation

$$y = \frac{h}{\sqrt{\pi}} \cdot \epsilon^{-h^2x^2},$$

where x and y are rectangular co-ordinates and $h =$ parameter of the curve. Riedel tabulated the heights of nearly 4,000 schoolboys of various ages, and found that the variations in height observed for each age were, for all intents and purposes, just what the mathematician predicted. Other investigators have confirmed this and have extended the scope of the equation, applying it to variations in weight, chest measurement, etc.

The index of variability, or standard deviation denoted by the letter σ, is equivalent to a determination of the point on the actual frequency curve, where it changes its curvature on either side of the mean. For example, if the curve showing the number of individuals of a certain age having a certain height, for instance, be plotted it will cut the theoretical curve at various points. σ is a measure of this divergence.

The coefficient of variability (Table LXXXIV.) is obtained by dividing σ by the mean and, for convenience, multiplying by 100,

$$i.e. \quad C = \frac{\sigma}{M} \times 100.$$

TABLE LXXXIV

Coefficient of Variability in Man at Various Ages

Age (yrs.).	Height C.	Weight C.
0	6·5	15·66
5	4·1	11·5
10	4·3	11·6
15	5·5	15·3
20	3·6	10·8

From this, we see that the coefficient of variability tends to decrease when the rate of growth decreases and tends to increase again when rapid growth restarts in the "teens."

(*b*) **Sex** introduces a phase difference in the rate of growth. Girls run through the various phenomena of growth at a more rapid rate than boys.

(*c*) **Difference in race**, even under similar climatic conditions, has a profound effect on the final *result* of growth—*i.e.* on total stature, weight, form, etc.,—but seems to have little or no effect on the *rate* of growth. The little Jap increases in size year by year at the same rate as the tall Norwegian. *The rate of growth is a specific phenomenon governed by factors deep rooted in the composition of the organism.*

2. External Factors.

Quite apart from these more or less normal variations due mainly to hereditary influences, there are various external factors which have a modifying effect on the rate and amount of growth.

(*d*) **Temperature.** As we have seen previously, all chemical and physical reactions respond to alterations in temperature by an alteration in velocity. In the terminology of van't Hoff, it may be said that if x is the temperature coefficient of a reaction, and the temperature of the reacting mass is raised n degrees, the consequent alteration in velocity will be as x^n. Usually the interval taken (*i.e.* n) is 10° C. and x is written as Q_{10}. For most chemical reactions Q_{10} is $= 2$. This may be taken to mean that for an increase of temperature of 10° C. the velocity of the reaction will be doubled. Van't Hoff noticed that, at low temperatures, very high temperature coefficients were obtained—in some cases Q_{10} reached the value of 5 or 6. Most physical reactions, as we have seen, differ from most chemical reactions in having a *negative* temperature coefficient, *i.e.* their rate is decreased by an increase of temperature. The various reactions which are manifested as growth are some chemical and some physical, and it is, therefore, somewhat difficult to apply the van't Hoff law to this phenomenon. Moreover, as pointed out in the earlier pages, Errera extended the principle of Le Chatelier by stating that—*every physiological process causing change, by its very action, set in being other reactions to inhibit change* (pp. 9 *et seq.*). It is, therefore, a difficult matter to interpret the figures obtained for the influence of temperature on the velocity of growth in animals.

Hertwig's classical work on the rate of growth of the tadpole illustrates the type of result got in this line of research. He found, for instance, that at 10° C. the tadpole took 6·5 days to reach the same stage of development that at 20° would have taken two days, *i.e.* the two rates are as $6{\cdot}5/2 = 3{\cdot}25$. Using the equation given above and putting $n = 10$,

$$x^{10} = 3{\cdot}25,$$

i.e. $$10 \log x = \log 3{\cdot}25 = 0{\cdot}5119.$$

Therefore $$\log x = 0{\cdot}05119,$$

and $$x = 1{\cdot}125.$$

The temperature coefficient for the development of the tadpole is thus $(Q_{10}) = 1{\cdot}12$. That is to say, if it takes t days at a certain temperature $\theta°$ (between 10° and 20°), for a certain amount of growth to take place, then it will take $t \times 1{\cdot}12^n$ days when the temperature has fallen to $\theta - n°$ C.

(*e*) **Climate.** The various meteorological conditions—temperature, relative humidity, nature of soil, etc., which are all included under the term climate—undoubtedly exercise an influence on animal and vegetable growth. The effect of relative humidity on plant growth has been exhaustively studied and conclusions have been drawn as to the concentration of moisture at each temperature which best promotes the growth of specified plants. It is more difficult to get statistics correlating animal growth with the various climatic factors. In order to study biological problems like this experimentally, one must have the power of altering the component factors one at a time and noting the results.

(*f*) **Seasonal variation.** Indubitable evidence is available to show that the growth-rate of the lower animals is subject to seasonal alterations. There are indications that positive and negative variations occur in man in summer and winter respectively (Table LXXXV.).

TABLE LXXXV

GROWTH IN HEIGHT OF GERMAN MILITARY CADETS IN HALF-YEARLY PERIODS (DAFFNER)

Number observed.	Age.	Increment in cm.	
		Winter, 6 months.	Summer, 6 months.
12	11—12	1·6	2·3
80	12—13	1·5	2·9
146	13—14	2·0	3·0
162	14—15	2·5	3·5
162	15—16	2·3	3·0
150	16—17	1·9	2·3
82	17—18	1·2	1·5
22	18—19	0·8	0·9
6	19—20	0·4	0·4

Other investigations (West Point, Sing-Sing, etc.) do not yield such a marked seasonal variation.

(*g*) **Diurnal variations.** Both weight and height vary in the course of 24 hours. The weight is lowest in the morning before breakfast, and is highest after supper in the evening. This may be accounted for by the fact that the weight of food eaten is greater than the weight of excreta. On the other hand, stature decreases during the day by from 1 to 3 cm. This trifling shortening is attributed to compression of the intervertebral discs, curving of the spine and depression of the arch of the foot. Measurements for comparative purposes should, therefore, always be taken at the same time of the day—*e.g.* before breakfast.

(*h*) **Nutrition.** It is obvious that, if the animal does not get an adequate supply of the material to be built into tissue, and of the energy necessary for these processes, the work will be done slowly and badly. In man at least, this only applies to increase in girth and weight. Growth in stature seems to be specific and is almost independent of the quality and quantity of the food available. In the lower animals, while decrease in growth is conspicuous in underfed animals, one may also detect a clear falling off in the rate of increase of length (cf. Table LXXXVI.).

TABLE LXXXVI

COMPARATIVE WEIGHTS AND LENGTHS OF FULL FED AND UNDERFED RATS

Age (weeks).	Length of body.		Weight.	
	Full fed.	Underfed.	Full fed.	Underfed.
0	48 mm.	48	5·4 gm.	5·4 gm.
6	128	98	42	24
10	173	100	150	25

The underfed rats were given food of just sufficient energy content to provide for their maintenance.

It is well known that quite apart from its energy content, food for growing animals must have certain of the amino acids in its make up. These are the building stones or units of which the complete organism is constructed. It has been shown that if animals are deprived, say, of the amino acid *lysin*, their growth is inhibited. On the addition of this amino acid to their diet, not only is growth restarted but leeway is made up and a normal growth is produced.

Certain much studied but little known *accessory factors* are absolutely essential constituents of the diet of the growing child.

Of their chemistry a little is known—of their physics or of the mechanism of their action nothing positive can be said (Chap. XIII.).

(*i*) **Social position.** The children of the well-to-do are generally markedly heavier and slightly taller than those of the working classes. Quite apart from any possible underfeeding of the latter, one must take into account the more or less sheltered life of the former and their freedom from those influences which tend to put the burdens and responsibilities of the adult on the child of the lower classes at a comparatively early age.

(*j*) **Endocrinetes.** Considering the factors which influence the rate of growth, and keeping well in mind the unthinkable complexity of the polyphase solution of colloids and crystalloids composing the animal body, one can hardly be surprised that so little is known of the mechanism of growth. In some way, the various alterations in size and shape are interrelated and regulated through the blood and through the nervous system by various secretions from endocrine organs. Growth in length is associated with the secretion of the pituitary gland. Any alteration in this gland causes alteration in the performance of other endocrine organs, *e.g.* the gonads and thyreoid. The growth of cartilage and bone are profoundly modified by alterations in the output of the thyreoid gland, while the gonads, and in early life, the thymus, control both growth and development, again by processes of which the mechanism, from a physico-chemical standpoint, is quite unknown.

IV. The Energy of Growth.

It is generally believed that young animals require much food *because they are growing.* That this is not quite correct has been shown in Chap. XXXII., where we saw that the young animal, because of its large surface compared to its mass, *lost heat* most rapidly. To prove this we need only examine the metabolic balance sheet of the child. Camerer gives the following composition of a new-born child (in grams):

Total weight.	Protein.	Fat.
2,820	320	348

Now, as we have seen, in 180 days the child doubles its weight. It does not, however, do so by adding equal quantities of the material already present. In a child 180 days old, weighing 5,600 grams, there are 790 grams of protein and 733 grams of fat. Thus 470 grams of protein and 385 grams of fat are added. In Calories that gives $470 \times 5{\cdot}3 = 2{,}491$ Calories $+ 385 \times 9{\cdot}3 = 3{,}580{\cdot}5$ Calories = a total of $6{,}071{\cdot}5$ Calories for the period, or about

35 Calories per day. A child of that age has an intake on the average of about 500 Calories per day. That is, the energy used for growth amounts to about 7 per cent. of the total energy intake.

It has been calculated that each gram of infant's body substance has a value of 1·87 Calories. Thus, if the child adds 20 grams a day, it " fixes " $20 \times 1{\cdot}87 = 37{\cdot}4$ Calories per day, a result closely approximating to that just given.

Rubner has formulated the following law regarding the energy of growth.

Law of constant energy consumption. " The number of calories required to double the weight of a new-born animal of all species (except man) is practically the same in spite of the enormous differences in time taken in attaining the double weight." Analysis has shown that each kilogram of body substance contains about 113 grams of protein and 120 grams of fat having an energy value of 1,726 Calories. Experiment has shown that in building this up the animal uses about 4,800 Calories. Man is an exception, and requires six times this amount.

The growth quotient is the percentage of the energy intake which is " fixed " in the animal tissues. It is about 36 $\left(\frac{1{,}726}{48}\right)$ for all animals except man, who is able to " fix " only 6 per cent. of his energy intake.

The question now arises as to why growth stops. If it is an autocatalytic reaction, growth should stop when the process is balanced, *i.e.* when anabolism and catabolism mathematically cancel one another. According to Loeb, this happens when the organism has reached a certain size, or a certain number of cells have been formed. Rubner's second law, that of length of life, is suggestive rather of the cessation of positive growth after the cell had expended a certain amount of energy. It is certain that if growth is inhibited during a certain period, *i.e.* if energy which would normally have been expended on building up tissue is not used for this purpose, the whole " growing time " may be prolonged.

The healing of a wound, the regeneration of tissues and the growth of tumours, etc., bear a close resemblance to animal growth as a whole. They may be modified by the same factors, and investigation of the various processes involved has shed considerable light on the mechanics of growth.

Leo Loeb and his co-workers have made an extensive study of the healing of a skin wound. They found that if the skin is

removed from any spot, epidermal cells from the edge of the wound creep upon the denuded spot and form a covering layer—a surface-tension phenomenon. The stretching of the contents of the surrounding cells so produced, causes a rapid series of cell divisions, *i.e.* growth under stimulation of stress takes place (Chap. XVII.). That the cause of the increased cell division is the stretching of the cells is borne out by the fact that the larger the area to be covered (within limits) the greater is the tension and the more rapid the process of forming a new skin. When the skin is formed and the internal pressure is altered from one of stretching to one of compression due to the crowding of the proliferating cells, growth slows down to normal. It is noteworthy that, before this return to a normal rate of cell division can take place, distinct pressure must be exerted by the epithelial cells on one another, *i.e.* excessive formation of epithelial cells occurs. *Is it possible, from this and similar experiments, to consider that cell pressure is one of the limiting factors in growth?*

There are, as we have seen, two main epochs of growth, each followed by a slowing down of velocity. In the first case, in early life, the slowing down is temporary. This may be correlated with (*a*) the fact that the increase in weight during this period is due in great part to a deposition of fat which is absorbed during the subsequent period of slower growth, and (*b*) to the changes in glandular functions, etc., which usher in the second period of very rapid growth. This final period is followed by a complete arrest of positive growth. The increased weight is due to muscle and organ building—protein is laid on and the percentage of water decreases. No further change resulting in increased metabolic activity takes place after this. Cell pressure now developed is not relieved.

The physical chemistry of negative growth will be considered in a later chapter.

V. Growth and Form.

One cannot leave this subject without a brief reference to the relationship existing between growth and form. *Form is determined by the specific rate of growth in various directions; i.e.* as D'Arcy Thompson puts it, form is a function of time. If a spherical organism grew symmetrically, its form would not alter, but because of its complexity, growth is not uniform in all directions. There are structural differences in protoplasm which set up unequal resistances to growth. One part may be more viscous than another, or may have a higher surface tension and so on. Although it has been pointed out that the presence of external

resistance acts as a stimulus to growth, it has also been said that internal resistance arrests growth.

Bohn propounds the following four laws relating growth and form in plants, and they may be applied to animals with some measure of justification :

(1) *Law of Vectors.* A *vector*, in distinction from a scalar phenomenon, is one representable graphically by a line of known direction and definite length, *i.e.* a conception of a change of magnitude with time. "The principal forces of growth are directed along axes offering a geometrical disposition."

(2) *Law of Depolarisation.* "When growth becomes exaggerated in a certain direction, a force is developed in the living organism which tends to oppose the growth," *i.e.* Errera's Rule, *q.v.*, or the ordinary law of balanced reaction.

(3) *Law of Axial Repulsions.* "When secondary axes are borne on the principal axis of a plant or animal, a reciprocal repulsive force is developed between the principal axis and each secondary axis."

(4) *Law of Compensation.* "When an axis branches in one plane there is a tendency for the re-establishment of the destroyed bilateral symmetry."

To summarise :

1. Growth is a balanced reaction having the mature organism for an end point.

2. The organism grows at a definite rate which is, at any moment, proportional to the amount of growth yet to be made.

3. The rate of growth is not uniform throughout but is specific for each epoch of life.

4. The growth in each epoch proceeds at a rate corresponding to an autocatalytic reaction.

5. Various factors which influence chemical and physico-chemical reactions, influence the rate of growth.

6. If the rate of growth is arrested in any epoch, the length of time spent in that cycle is prolonged—so that the amount of growth characteristic of that epoch is accomplished.

7. Form is a function of growth.

CHAPTER XXXVI

DEVELOPMENT

". . . I compared the cell-growth, by which Nature builds up a plant or an animal, to the glass-blower's similar mode of beginning,—always with a hollow sphere, or vesicle, whatever he is going to make."

OLIVER WENDELL HOLMES.

OCCURRING simultaneously with increase in size, are changes in external form and internal structure—the organism develops. Mainly through the brilliant researches of J. Loeb and his school, some light has been thrown on this seemingly mysterious and apparently inexplicable process. The changes taking place are most readily perceived when the transparent eggs of the echinoderms are used as the material on which to experiment, and consequently, our ideas of the processes involved in mammalian development are largely derived from the study of processes in the lower aquatic animals which may or may not be quite analogous.

Cell Division.

Cell division is the most general of the specific functions of living protoplasm, and it is the basis underlying the differentiation of the comparatively simple structure of the egg into a more complex organism. The division of a cell is a necessary consequence of its increase in volume. The metabolic activity of the cell is a function of its effective surface, *i.e.* its surface must be of such a size compared with its volume that an adequate supply of oxygen can reach the centre of the cell and that all the by-products of cellular activity can be eliminated with sufficient rapidity. A freely suspended unicellular animal is spherical. Its surface-volume ratio is 3/1. Doubling the radius increases the surface four times while increasing the volume eight times, *i.e.* $S/V = \frac{3}{2}$, *i.e.* the effective surface has been halved. *The immediate result of decreasing the specific surface to a value below the minimal effective value is to decrease the supply of oxygen to the centre of the cell and to cause a heaping up of carbon-dioxide and other products of metabolic activity.* This has, at least, two effects—(*a*) The process of development is retarded (law of balanced reactions), (*b*) The protoplasm becomes acid. The effect of acid

on an alkaline gel-emulsion has already been considered and may possibly be the cause of cell division. We have, however, to inquire into the reason for the *symmetrical* division of the cell.

Some cells divide directly without showing mitotic figures. After a change in the distribution of free energy manifested by a division of the nucleolus into two separate nucleoli, the whole nucleus is divided equally into two daughter nuclei. This separation is followed by the formation of a cell membrane between the two nuclei dividing the entire cell into two equal and similar portions.

Usually, however, cell division is accompanied by a complex but regular series of changes in the distribution of the nuclear chromatin. These *mitotic or karyokinetic* changes are dependent on the *bipolarity* of the cell. Morphologically the polarity of a cell refers to a symmetry of visible structure about a particular axis. For instance, a line, drawn through the centres of nucleus and centrosome, symmetrically divides a typical resting cell and may be considered as its axis of polarity. *This symmetry of form is an indication or expression of a symmetry of free energy.*

In a bipolar cell there are two " poles " or centres of force, and the axis of symmetry must divide the field of force equally about these poles.

Typical fields of force may be plotted by scattering iron filings on a sheet of glass resting on the pole (monopolar field) or poles (bipolar field) of a magnet. The filings set themselves along the lines of force, each little filing becoming polarised and exerting an influence on adjacent filings. (This " carding out " under the influence of stress was dealt with in Chap. XVII.) In addition to the strength of the field and the direction of the force, the movement of particles under its influence depends on the friction of the contiguous matter and on the chemical nature of the particles themselves. Friction prevents the filings from collecting in mass round the poles while the specific inductive capacity (*q.v.*) or " permeability " of the particles is a measure of the influence exerted by the " force " on the particle. In the case of magnetic force, the specific inductive capacity of iron is high while that of bismuth is low. Iron filings will, therefore, be attracted towards the poles and will tend to lie on the lines of stress. On the other hand, bismuth filings are polarised in a sense opposite to that of the adjacent field. They are forced by the incidence of stress to move (or because of friction, to tend to move) from the stronger to the weaker parts of the field and thus take up positions as far from the poles as possible. *In general, a body placed in a field of force will tend to move towards regions of greater or less intensity of stress according as its " permeability " to the particular form of energy in question is greater or less than that of the surrounding medium.*

The introduction of an aggregate of high permeability into the field of force makes considerable changes in its configuration. Suppose a small heap of filings were placed in the magnetic field already referred to, so that it lay somewhat out of the interpolar axis but on the equatorial axis, the result would be to provide an " easier path " for the lines of force. It is

obvious that, within certain limits, a longer path through a more permeable mass would be more advantageous than a shorter path through a less permeable medium, and so many of the lines of force would be "short-circuited" through the heap of filings. If, moreover, the heap of filings were free to move, they would be drawn *en masse* into the field of force until a point of equilibrium was reached. This resting place would depend for its position on the relative "permeability" of the filings in heap and the filings distributed over the field.

We have dealt with a magnetic field of force because it is easy to demonstrate and may be readily modified experimentally, but our remarks are applicable to any field of force. A simple experiment, due to Leduc, shows that the lines of stress set up by diffusion may be made manifest. A layer of salt solution is spread over a flat sheet of clean smooth glass, and on top of this is placed a small drop of indian ink or blood. A drop of a hypertonic solution of common salt is placed on either side of this central drop. In a short time the pigment seems drawn out into threads (μίτος) stretching between the centres of the two salt drops, so making a figure exactly the same as that formed by iron filings under bipolar magnetic influence.

The Bjerknes phenomenon demonstrates the applicability of this treatment to the stresses and strains set up in a fluid as the result of movement in it. Bodies synchronously vibrating or pulsating in a liquid medium attract or repel one another according as their oscillations are identical or opposite in phase. That is, a bipolar field exists which may have, like a magnetic field, similar or dissimilar poles. In such a field of force currents are set up in the fluid (hydrodynamic lines of force) and any particles in suspension, if lighter than the fluid, act like the iron filings, if heavier like the bismuth filings above. Moreover, the lines of force set up by identically pulsating (attractive) bodies are exactly similar to those produced by similar (repulsive) magnetic poles, and *vice versâ*.

The first stage in cell division consists in the division of the centrosome into two equal parts which draw away from one another. A field of force is set up between the two centrosomes and threads of those cell constituents which are more "permeable" to the form of energy existing, are carded by the incident stress into a figure closely resembling those mentioned above. On the "outer" side of the centrosomes can be seen starlike radiations (astral rays) recognisable as indications of incomplete lines of force which run externally to those stronger interpolar lines which constitute the achromatic spindle.

The chromatin of the nucleus is drawn into a continuous thread, making a skein, which is then broken into short V-shaped lengths, the *chromosomes*, of which there are 24 in human somatic cells. These chromosomes become arranged round the equator of the achromatic spindle with the apex of the V pointing to the centre. This completes the first or prophase.

The second phase of karyokinesis commences by the longitudinal splitting of each chromosome (metaphase). One longitudinal half of each chromosome then passes (anaphase) to each pole of the

spindle, so that we have, in all, 48 chromosomes, 24 at each pole. This completes the second and third phases of division.

The fourth or telophase consists of retrogressive changes, that is, the chromatin of the daughter nuclei is formed by the fusion of the chromosomes of each group to form a skein at each pole, which then becomes transformed into the karyomitome characteristic of the nucleus. At this time, constriction of the protoplasm of the cell takes place in the neighbourhood of the equator of the spindle; the spindle disappears, and finally each daughter cell, with its full complement of chromosomes, becomes an entity.

The nucleus is composed of material of fairly high "permeability" and, therefore, may be expected to travel towards the equatorial axis. This is found to be so. In some cases the nucleus is wholly, and in other instances it is only partially, drawn into the field between the centrosomes. Differences, too, exist in the relative development of asters and spindle which are capable of explanation by analogy to the magnetic model. If, in the experiment with iron filings, the field were surrounded by an iron ring, the majority of the lines of force would pass round by the ring. That is, the interpolar lines would be slight and the extra-polar rays would be heavy. Similarly, we may correlate a mitotic figure having good astral rays and a poor spindle with a marked "permeability" of the surface of the cell.

One would be entering the realms of pure hypothesis if physico-chemical interpretations were attempted of the various stages of karyokinesis. The constitution of protoplasm—vaguely stated as an emulsion of various lipoids in a complex protein-water emulsoid with various crystalloids in solution or adsorbed, presents excellent opportunities for the theorist to draw parallels between certain manifestations of force in living things and in dead matter. The mechanisms underlying these processes are as yet unknown. The processes themselves, like all other changes in matter, are accompanied by changes in electrical potential. These changes are measurable, and are not constant, but fluctuate (even reversing in direction) at epochs coinciding with phases of development.

Cause of cell division. We are now in a position to consider the actual cause of cell division. To state *why* the cell must divide—to argue from a surface-volume ratio—is to presuppose a cell consciousness or to postulate an external directing force—both alternatives being outside the domain of physics. The use of the final cause, or the argument that division is of obvious advantage to the cell, sheds no light on the mechanism involved. Consideration must be given to the forces at work in the cell. Further, experiments such as the much quoted one of Brailsford Robertson,

where an oil drop is divided by the imposition on it of a thread soaked in alkali, are not very illuminating. The energy of cell division is not external to the cell, but depends entirely on a redistribution of forces *inside* the cell.

We have seen that, as a result of the diminution of specific surface with growth, metabolic processes are retarded and acid by-products tend to accumulate. It is obvious that at the centre of the cell, *i.e.* at the region most distant from the surface, these changes due to maloxidation will be most marked. This decrease in metabolism is accentuated by the fact that the nucleus, which is always in the centre of the field of energy of the cell and usually near the centre of the cell material, is the seat of the most rapid oxidation changes in the cell. It will, therefore, show the effects of the lack of oxygen at a very early stage. It follows from this, that the intrinsic energy of the fluid at the centre will either suffer an increase or a decrease. Everything points to the latter as occurring. Now, as surface tension is, as far as cell problems are concerned, a relative magnitude, we may say that the tension of the superficial layer of the cell which, on account of its proximity to the surface, remains practically normal, increases in close correspondence with the decrease of intrinsic energy of the central portion.

This increase is still further accentuated by the increase in the relative " permeability " to energy of the surface of the cell, which, as we have seen, causes the development ot radial lines of force. The high tension of the surface of the egg operating on the central region of low intrinsic energy causes the material in the centre to be dispersed into the cytoplasm of the cell. These dispersed particles first set themselves in a neutral position, *i.e.* on the plane of segmentation—cf. iron filings in a magnetic field of force between two similar poles. The position of the nucleus determines the first plane of segmentation, since apparently nuclear division precedes the visible division of the cytoplasm of the egg. In other words, the plane of nuclear division becomes the plane of segmentation of the whole cell. The nuclear matter, as manifested by the chromosomes, gradually sets in the lines of force between the centrosomes and slowly separates into two equal portions which ultimately form the two nuclei. Hence we have a spherical cell which is capable of division into two exactly similar portions. It is, therefore, manifest that each potential segment, because of this similarity of structure and energy content, will repel the other half and, according to the ordinary laws of stresses and strains, will cause division in the plane of symmetry. The high tension of the cell surfaces will ensure the continuity

of the surface-membrane of each of the daughter cells. These daughter cells adhere to one another but do not coalesce. From this it is inferred that the cell membrane is insoluble in the surrounding medium and in the cytoplasm as well. (It has been

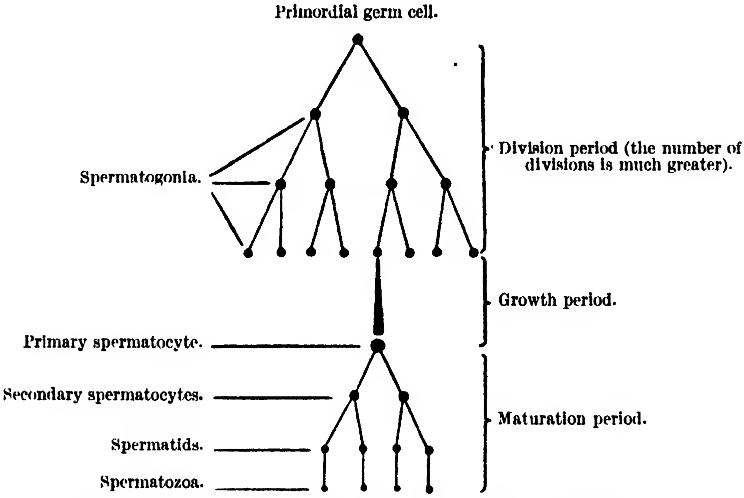


FIG. 99.—Scheme of the Processes involved in the Maturation of Spermatozoa.

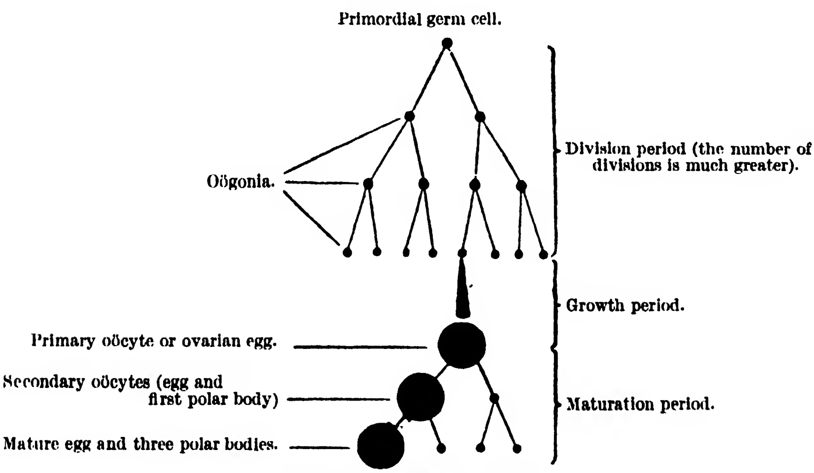


FIG. 100.—Maturation of Ovum.

proved experimentally that hanging drops coalesce if their surface films are soluble in the drops themselves, while they separate if the film is soluble in the surrounding medium.)

Maturation of Germ Cells. The cells of the body may be divided into somatic or true body-cells and gonads or reproductive cells. Certain of the cells of the gonads become germ cells. The process

of mitosis follows a path somewhat different in the germ cells from that of the somatic cells. The primordial germ cell, after dividing in the same manner, as we have seen somatic cells do, undergoes a process of maturation which is an additional series of divisions resulting in cells with only half the number of chromosomes that the parent cell possessed (Figs. 99 and 100).

Fertilisation. This is the union of a male cell and a female cell, whereby incidentally the full number of chromosomes is restored and a further series of cell divisions instituted.

The unfertilised ovum is a moribund body which disintegrates more or less rapidly. If, before disintegrative processes have become apparent, the egg undergoes fertilisation, destruction is stayed. The fertilised egg develops, grows and becomes differentiated into various structures. *This process of differentiation of protoplasm is an orderly one, taking place always in the same manner and being modified always by the same conditions.*

On the entry of the spermatozoön, some change in the free energy of the egg must take place. The egg is no longer static but becomes endowed with dynamic force. In order to discover the underlying physico-chemical change, Loeb attempted to induce development of unfertilised eggs by alteration of the environmental conditions. No change in a system in equilibrium can take place unless the relative amount or incidence of the free energy of the environment is first altered. The two series of changes—external and internal—are cause and effect. This is merely a restatement of the *Law of Inertia.* The entry of the spermatozoön alters the balance of free energy between egg and environment. Loeb attempted to bring about the same result by altering the free energy balance between environment and egg. He found that two separate and distinct changes took place after fertilisation, viz., membrane formation and development. These involve totally different physico-chemical reactions. Membrane formation is not followed necessarily by development.

I. Membrane Formation.

Loeb found that all those substances or agencies which can bring about hæmolysis (Chap. XXII., p. 318) also induce membrane formation. The best agent for this purpose is dilute butyric acid. Immersion of sea-urchins' eggs (unfertilised) in sea-water containing about 5 per cent. of $N/10$ butyric acid for 2 to 4 minutes brings about typical membrane formation. This membrane is tough, and is separated from the egg-substance by a layer of more fluid material.

Examination of the performance of the other cytolytic sub-

stances makes manifest the mechanism of the change brought about by their agency, and its similarity to that caused by butyric acid or other fatty acid. The former all lead to the abnormal production of acids *in living protoplasm*, and these acids produce, as a secondary effect, the physico-chemical change now under consideration. These cytolytic substances are, as we saw in Chap. IX., just those substances which break emulsions. Egg protoplasm is an emulsion very rich in fat, and it is obvious that the breaking of such an emulsion would lead to the setting free of protein and would probably change the nature of the complex from aqueous protein-in-oil type to an oil-in-aqueous-protein type. The protein and lipoids carried to the surface and coming in contact with sea-water would readily be adsorbed and form a membrane (Chap. XI.).

Eggs undergoing artificial parthenogenesis quickly show disintegrative changes unless means are taken to confine the cytolytic effect to the surface. This is provided for in some cases (*e.g.* starfish and certain annelids) by the specific nature of the proteins in the cortical layer. The diffusion of the acid causes them to alter in electrical state. They imbibe water, swell up and develop normally.

II. Exosmosis.

In most cases, however, unless a second alteration is made in the bathing medium, the egg will either not develop at all, or will die at some intermediate stage. It is known that after fertilisation the electrical conductivity of the egg is increased for 15 minutes or so. This may be interpreted as a sign of increased permeability of the membrane to surrounding salts, or it may, with equal justice, be accounted for by a withdrawal of water with a consequent increase in the concentration of electrolytes. During this period of increased electrical conductivity the eggs readily undergo plasmolysis if placed in a solution of cane sugar. Unfertilised eggs, of course, do not show alterations at this early stage in electrical conductivity, nor are they so easily plasmolysed. This may be taken as a confirmation of the second hypothesis, viz. that water is removed by exosmosis and, consequently, the concentration of electrolytes in the egg is increased. In order to induce an exosmotic flow, the membrane must be impermeable to sugar. If the membrane had increased in permeability during fertilisation the fertilised egg would probably be less easily plasmolysed than the unfertilised egg (see Chaps. V. and XI.) In addition, it is probable that an alteration takes place in the ratio of the free to the bound water in the egg, and that

adsorbed electrolytes are set free, so increasing the electrical conductivity of the cell. Whatever be the actual physico-chemical process brought about by the entrance of the spermatozoön, the result has been imitated by simple physico-chemical means.

If, after removal from the butyric acid bath (or other cytolytic agency), the egg is placed in hypertonic sea-water for about half an hour and then returned to its normal environment it will, in all likelihood, reach maturity. That is, *not only does artificial membrane formation initiate the processes of development, but it starts, at the same time, processes which ultimately lead to the dissolution of the organism.*

These latter activities may be, for a time, suspended, by a short exposure to a hypertonic solution.

Loeb has proved that the withdrawal of water is merely the trigger setting off a series of chemical as well as physical changes. Attention has been repeatedly drawn in previous pages to the fact that while most physical processes have a low, or even a negative temperature coefficient, most chemical reactions have a high ($Q_{10} = 2$ or more) temperature coefficient. This worker found that, at a temperature of 5° C., the eggs had to remain in the hypertonic solution for at least 210 minutes. The time of exposure was decreased to 40 minutes when the temperature of the solution was raised to 15°. Therefore, the temperature coefficient for this process is

$$\frac{210}{40}, \; i.e. \; Q_{10} = 5.$$

Hence, superimposed on the physical process of exosmosis are secondary chemical reactions initiated by it.

Spermatozoön. Attempts have been made to determine what part the spermatozoön plays in the process of fertilisation. Brailsford Robertson has extracted a substance, *oöcytin*, from the testicles of the sea-urchin which produces membrane formation. The question then arises—"Is this substance a catalyst speeding up some slow change or does it counteract some obstacle to development?" To the first part of the question a negative answer can be given. The velocity of the process of development is not catalytic. It does not follow Schütz's law and vary in velocity with the square root of the concentration. If two spermatozoa are caused to enter an ovum, the rate of segmentation should increase by 1·4 (*i.e.* $\sqrt{2}$) times, if the process were catalytic. The rate, as a matter of fact, is unaltered by the introduction of additional spermatozoa. Therefore, *the spermatozoön does not contain a catalyst for developmental processes.*

Loeb is inclined to believe that the spermatozoön removes from the egg a substance or condition which inhibits or prevents the process of development.

On the other hand, it is conceivable that the entry of the spermatozoön increases the *free energy* of the now fertilised ovum. The potential energy of the system cannot be utilised without the employment of a small quantity of free energy. This quantity of free energy may be extraordinarily small as long as it is sufficient to start the series of reactions which, once started, are autocatalytic.

One result of the entrance of an effective spermatozoön into an ovum is an acceleration of the processes of oxidation, *i.e.* metabolism begins, and the various phases of development can be followed by the same calorimetric methods (direct and indirect) adopted in the study of the energy exchanges of the mature organism.

Differentiation. The mammalian ovum is holoblastic, that is, undergoes complete segmentation, and forms a mulberry-like mass of cells which divides into two sets, viz. a group of large central cells and a layer of small cells surrounding these. That is, there is now an *unequal* division of the cell material. Three zones can be recognised in all the cells up to this stage. These three zones, viz. (*A*) a clear cap at one pole; (*B*) a zone with a pigmented surface; and (*C*) a large unpigmented zone, each gives rise to a definite part of the developed egg. Thus, up to this division every constituent of the original egg is present in the segments in the original proportions. The division now is equatorial, and cleaves the cell-mass unequally. Four cells are formed containing little or no *A*, and the other four cells contain only a trace of *C*. These latter form, at the next division, four very small cells called micromeres mostly of *A*, and four larger pigmented cells (intestinal cells). Eight cells (*ectodermal* cells) are formed from material which is mostly *C*, but contains some *B*.

The cell division proceeds and the tiny cells all gather at the surface of the egg—surface adsorption. Soon after the tenth division, when the number of cells is theoretically 1,024, the processes of invagination and differentiation begin.

Organo-genesis. Various parts of the egg give rise to various organs. The same organ is formed always from the same part. This means that the apparently homogeneous protoplasm is heterogeneous, *i.e.* contains colloidal matter in different parts of maybe a specific chemical nature—certainly in a specific physical state. One cannot, as yet, say why certain cells grow in certain directions or why certain organs should be evolved from certain

cells and only from these cells, but certain mathematical and physical phenomena have been observed in this connection.

If one postulates, in the first instance, the presence in the egg of regions denser than others, for example, one can imagine as a result unequal growth in various parts. Unequal growth sets up strain; and strain, as we saw in Chap. XVII., influences the external form and internal structure of organs. This can be demonstrated experimentally by building an artificial *blastula* of little pellets of dough containing different quantities of yeast. The unequal growth of the various pellets sets up mutual strains and produces a considerable folding and distortion of the whole (Roux).

Both the circulatory systems and the alimentary canal are evolved from tubular structures, and it is suggestive to find that certain phenomena of development are mirrored in inorganic tubes. For instance, a thin test-tube very often cracks in a spiral way. The more homogeneous and isotropic be the glass the more even and regular will be the spiral. That is, the track tends to follow the shortest course *on the surface of the tube* between the point of origin of the crack and a point diametrically opposite—a ring formation. Generally, however, the ring winds into a helicoid form and is continued. This helicoid geodetic is shown in the coil which stiffens the tracheal tubes of an insect and is apparent in the growth of the intestine. Carey plotted the positions of the cells showing mitosis in the intestinal epithelium at various levels. He found that they formed a left-handed helix (5 per cent. right handed) having its base towards the rectum and its apex towards the ileocæcal valve. Further, the mitotic figures were few near the base and increased in number as the apex was approached. From this he inferred that growth was from below upwards and followed a helicoid path.

One must consider that a definite mechanical action is due to incidence of stress and that similar results under similar conditions are good evidence of the imposition of similar forces. The homogeneous hollow glass tube splits spirally, which can only be interpreted as the spiral path being the line of least resistance in a hollow cylinder. We can apply this with justice to the growth of intestinal epithelium.

A growth tension applied helically must lead to torsion in a structure like the intestine where there are layers of material growing at different rates, and one could present a very plausible diagram of forces to explain the twisting and looping of the gut. Similarly and inversely, dilatation, *e.g.* stomach formation, would produce alterations in the direction of the lines of growth leading to alteration in the arrangement, say, of the muscular fibres.

Energy of Development.

Interesting observations have been made of the amount of energy used by a developing organism. Tangl determined the energy content of fresh laid eggs and compared that amount with the energy value of the embryo and yolk found in the shell at the moment of hatching. He reported that each gram of chick had been formed at the cost of the energy represented by 658 small calories.

Further, 35 per cent. (32 Calories) of the total chemical energy of the fresh egg is deposited in the tissues of the young embryo; 48 per cent. (44 Calories) is found to a large extent in the abdomen of the chick as a store of potential energy to be drawn upon during early life; while the balance—17 per cent. (16 Calories)—has been spent in the development of the chick. That is, about one-sixth of the total energy of a hen's egg is required for the work of elaboration of the tissues of the chick, which tissues contain one-third of the original energy of the egg.

CHAPTER XXXVII

DEATH AND DISSOLUTION

It is easy to show that these differences in temperature which are required to secure organic liquids from ultimate change depend exclusively upon the state of the liquids, their nature and above all upon the conditions which affect their neutrality whether towards acids or bases." PASTEUR.

IT has been said that death is a necessary stage in the process of development. Rubner considers that death takes place naturally after the organism has utilised a certain amount of energy per kilogram. His second law, that of " length of life," is as follows, " *The amount of energy consumed in a kilogram of living protoplasm from maturity to death is constant for all animals* (*and equals* 191,600 *Calories*), *except in the case of man, who uses up four times as much.*" Be that as it may, and no adequate proof of its truth is offered, it does not serve as a guide to any reasonable physico-chemical explanation of the process. An inorganic piece of machinery will last an indefinite time provided it is kept in repair and parts are renewed before they have become too much worn. As long as suitable energy, etc., is supplied the machine will run. The human machine, with its large repair staff always " on the spot " and with plentiful supplies of material and energy, begins to show signs of failure after 35 or 40 years of life. The curve of growth, development and efficiency each shows a maximum and then decline sets in.

Length of life is *specific* for each species and seems to be related to the time taken by the animal to reach sexual maturity. With that consummation, changes take place in the whole organism leading, according to Loeb following Metchnikoff, to the (unavoidable) formation in the body of some toxin or, as more modern work suggests, to the inhibition of the formation of an endocrine secretion.

Death is followed by a more or less rapid dissolution of the body, a process whose mechanism is more easily followed. The lack of oxygen in the tissues leads, as we have seen, to the accumulation of acids. This, after death of the organism, occurs in *every* tissue, but it may also be demonstrated in cases where a particular organ or region of the body is deprived of its quota of oxygen. By the administration of certain drugs, *e.g.* anæsthetics, heavy

metals, phosphorus, potassium cyanide, oxidation processes are inhibited and acids accumulate. The first visible change after the inhibition of oxidation—general or local—is a "*softening*" of the tissues concerned. If water is available the involved cells swell and become cloudy accompanied or followed by a "*yellowing*" and the appearance of fat globules. The cells then tend to shrink and liquefy. These changes can be mimicked by the addition of a trace of acid to an oil-in-protein emulsion. The emulsifying colloidal proteins, under the influence of acid, develop an increased capacity for the imbibition of water. If water is available, the proteins swell and become extremely dilute and the emulsion is broken. The "*graying*" or cloudiness is due to the presence of colloids (globulins ?) which become less hydrated in an acid solution. The hydration of the one class of colloids and the dehydration of the other class leads to "*cloudy swelling*."

The breaking of the emulsion sets free the fat which is present, though normally invisible, in all cells. The tissue becomes yellow, and, as the pathologists say, "fatty degeneration" has become apparent. It must be understood that the fat made manifest by this process existed previously in the cell masked by its association with proteins, etc., in the emulsion. Its appearance at this stage of dissolution is not due to the conversion of protein or any other cell-constituent into fat as the name "fatty degeneration" might suggest. Careful analysis has shown that the total amount of fat in the cell has not increased.

As an emulsion has a much higher viscosity than its constituents, one might expect that the breaking of the emulsion would lead to a decrease in viscosity or softening of the tissue concerned. Further changes take place which make this loss of rigidity more marked and cause the ultimate dissolution of the protoplasm.

Almost coincident with the cessation of respiration, the endo-enzymes begin to accelerate the processes of hydrolysis of the tissues (p. 121). Under sterile and anaerobic conditions, the tissues may be converted into an almost odourless fluid—a process termed autolysis. Proteins are broken down to their constituent amino acids and, if autolysis is carried on sufficiently long, some of these acids may be destroyed. Instead of fat, autolysed tissue contains fatty acids and soaps. This self-digestion is a consequence of the lack of free oxygen in the tissues, which lack, as we have seen, causes the accumulation of acids. It has been shown that a very slight increase in hydrogen ion concentration so alters the tissue constituents that they are readily acted on by cellular enzymes.

In Chap. X. we mentioned the interesting fact that the enzyme which hydrolyses maltose builds up another carbohydrate, iso-

maltose, which it is incapable of breaking down. *In general, when a synthesis is brought about by an enzyme, the product is immune from being broken down by its builder.* But by the hydrating effect of acid these synthetic products are converted into isomeric forms which can be destroyed by the enzymes which originally formed them.

In addition to autolysis, micro-organisms present in the intestinal tract or otherwise entering the body from outside, play a large part in the dissolution of the organism. Putrefaction is readily distinguished from autolysis by the odour of the products of its action.

Just as the material composing the body returns to the earth to begin anew the cycle of life—passing from soil bacteria to plant, from plant to animal and from lower to higher animal—so the energy of the constituents of the body pass into the great cistern of unavailable energy, " waste heat," from which we are unable to draw supplies, but which by raising the level of the total cosmical heat energy ever so slightly, contributes to the well-being of all living things by raising, in imperceptible amounts, it is true, the level of metabolism.

CHAPTER XXXVIII

THE EFFICIENCY OF THE ORGANISM

By E. P. Cathcart, M.D., D.Sc., LL.D., F.R.S., Regius Professor of Physiology, University of Glasgow.

The consideration of the efficiency (*i.e.* the relation of the consumption of energy in the form of fuel to the output of energy in the form of effective work) of man in the production of external work is a question not merely of great physiological but of economic importance, as this factor plays an important *rôle* in the assessment of an adequate diet. Physiologically we are concerned with the abstract problem of the conversion of food energy into work—that is, the problem is simply the relation of the increased energy output during the actual performance of muscle work to the energy expenditure of a similar period when no work is being done. In the case of industry, armies, etc., the question is plain enough, but there are many factors both psychic and physical which qualify the answer: in other words, the types of work, the conditions under which it is performed and the personal qualifications of the performer all play an important part in the degree of efficiency with which the work is carried out. Hence it is very essential that the "net or *physiological*" efficiency be differentiated from the "gross, crude, or *industrial*" efficiency. The "net" efficiency may be defined as the value obtained by dividing the heat equivalent of the external effective muscular work by the increase in energy output of the body developed as the result of the work done. The "gross" efficiency, on the

TABLE LXXXVII

Heat output per min.			Heat equivalent of external muscular work per min. (425 Kgm. = 1 Cal.).	Efficiency.	
Work. (*a*).	No Work. (*b*).	Increase of work over no work = *a* − *b*. (*c*).	(*d*).	Gross. $\frac{d \times 100}{a}$.	Net. $\frac{d \times 100}{c}$
Cals.	Cals.	Cals.	Cals.	Per cent.	Per cent.
9·50	3·08	6·41	1·96	20·6	30·6
5·71	1·14	4·57	1·06	18·6	23·2

other hand, is the value obtained as the result of dividing the heat equivalent of the external effective muscular work by the total energy output of the individual during the period in which the work was done. The table on p. 495 will make the point clear.

It is obvious that the two efficiencies may give very different values. The gross efficiency, which is largely influenced by the amount of work performed during the day and the amount of time which is actually expended in doing work, as a physiological measure gives little or no information regarding the capacity of the human body for work, and certainly no conception of the possibilities in the way of the efficiency of the organism as a machine. The net efficiency, which is determined by the deduction of the maintenance quota from the work quota of the energy output, does give the actual increase in cost necessitated by the performance of the external muscular work and thus permits of the determination of the actual physiological efficiency of the organism.

In view of the fact that engineers and others have found it a comparatively simple matter to determine the efficiency of ordinary thermodynamic machines by the use of a simple formula $E = (T_1 - T_2)/T_1$, where T_1 is the absolute temperature at the source of the heat (the steam in the boiler in the case of an engine) and T_2 the temperature at the sink (the condenser of the engine), there has been a great temptation to apply the apparently almost universal rule of the Second Law of Thermodynamics to the living organism (Chap. IV.). It is, however, obvious from a very brief consideration of the above simple thermodynamic formula that the efficiency is simply a function of the difference of potential, the higher the temperature at the source and the lower the temperature at the sink the greater is the efficiency. Now the efficiency of the living organism has experimentally been shown to be high, probably over 30 per cent., a result which would necessitate an impossible difference of potential in the tissues. It is perfectly true that this difficulty has been appreciated, but it is not solved, except on paper, by positing minute points of enormously high temperature alternating with points of low temperature at intervals of a few μ (10^{-3} mm.). The mechanism of muscular activity, it is true, is not yet clear, but it may be stated with a considerable degree of certainty that, whatever the type of change which takes place, all the experimental evidence available points to the muscle not being a heat engine. The majority of workers now look upon muscle as a chemical machine which works at a relatively constant temperature.

On the purely experimental side much work has been done on

the determination of the efficiency both of isolated muscle and of the body as a whole.

If the organism be considered as a whole and its efficiency determined, it is found that, although it is high, it is never as high as the results which have been obtained experimentally with isolated muscle. This result is not to be wondered at when the methods of attacking the problem are considered. In the case of the isolated muscle, its position, the amount of work to be done and the mode and time of stimulation can all be accurately controlled, conditions which are, for the most part, lacking when the whole organism has to be dealt with.

Modern work has shown very considerable agreement as regards the degree of efficiency, as is shown by the following table :

TABLE LXXXVIII

GROSS AND NET EFFICIENCY OF THE BODY AS A WHOLE

Authority.	Efficiency.	
	Gross.	Net.
	per cent.	per cent.
Katzenstein (1891)	13–19	25·4
Sonden and Tigerstedt (1895)	17·3	27·4
Zuntz (1909)	—	28·0
Benedict and Carpenter (1909)	15·0	20·6
Amar (1910)	—	32·5
Benedict and Cathcart (1913)	—	21–33
Lindhard (1915)	—	25·0
Douglas (1920)	—	23–26

The outstanding difficulty in the assessment of the net efficiency is the selection of the proper base line for comparison. It is immaterial whether the work done be that of marching or mountain climbing, of turning an ergostat or a bicycle ergometer, the same difficulty crops up. As the bicycle ergometer has been most frequently used in the modern experiments it will be dealt with here.

In the determination of the mechanical efficiency with this machine no less than five base lines may be used though they are not all of equal value. In this type of ergometer, where the work to be done can be readily altered by increasing the resistance to be overcome, it is a comparatively simple matter to devise a wide range of experiments in which the effective muscular work can be varied. The only difficulty lies in the selection of the base line. If the work standard be taken as that of the subject sitting on the bicycle performing a definite measured amount of work,

in order to find the increased cost in energy caused by the performance of this work there may be subtracted :

(1) The energy expenditure during complete rest—the ordinary basal or standard metabolism.

(2) The energy output when the subject is sitting at rest in the saddle.

(3) The energy expended when the subject is sitting on the saddle, feet on pedals and his legs are rotated by mechanical means—internal or organic work.

(4) The energy expenditure when the subject is freewheeling, *i.e.* overcoming the ordinary resistance of the unloaded wheel with most or all of the concomitants of work of this type, sitting posture, internal friction of the legs, extraneous movements associated with cycle riding, etc.

(5) The energy expenditure involved in (*a*) the performance of light work compared with that of hard work, or (*b*) the increased cost of work done at slow and high speeds using the same load in each case.

When these various base lines are utilised experimentally it is found that there is a steady increase in the degree of efficiency. The average results are as follows :

TABLE LXXXIX

Base line.	Net efficiency. Average value.
(1) At rest . . .	21 per cent.
(2) Sitting . . .	Similar to (1)
(3) Internal friction .	27 per cent.
(4) Free wheel . .	30 per cent.
(5) Low to high speed .	30-33 per cent.

There is then a variation in the determined efficiency of approximately 10 per cent., and it is a moot question which base line should be selected. Lindhard maintains that the most reliable result is obtained when complete rest or rest in the riding position is adopted as base line, but there is much to be said in favour of the adoption of other base lines in which movements which play little or no part in the determination of the efficiency are eliminated. As the main object is to determine the efficiency of the body performing a definite act it has been suggested that the best result will be obtained when the various activities associated with the determination of the energy output both of the base line and of work are more or less comparable, that is, where the extraneous

muscular motions incidental to riding with weight are common to both determinations. Such a comparison is that obtained when there is a change from a moderate to a heavy load. As will be noted from the above summary of efficiencies the average efficiency under these conditions is about 30 per cent.

There is a certain amount of evidence available which would suggest that the degree of efficiency obtained varies with the groups of muscles used in performing the work. The efficiency of muscles less commonly in use than the leg muscles is somewhat lower, flexor groups may differ from extensor groups, etc. The state of training, too, probably influences, although apparently not very markedly, the degree of efficiency. And finally, some workers maintain that the efficiency may also be, to some extent, dependent on the nature of the diet. Macdonald maintains that the efficiency of muscular work is a function of body mass.

Greenwood, who has carefully analysed the data obtained by many of the workers, has come to the conclusion that although *as yet* no law can be formulated connecting heat production and work performance, within fairly wide ranges, simple formulæ of linear regression do describe the relations subsisting between heat production, body mass and work performance, with an accuracy sufficient for such purposes as roughly computing the energetic needs of workers doing the kind of work studied.

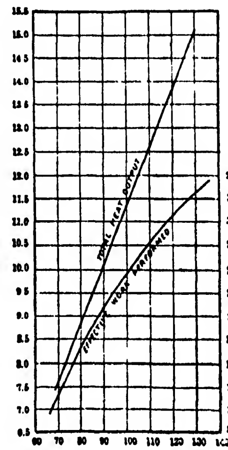


Fig. 101.

In addition to the above-mentioned factors which influence efficiency there are certain others connected with the performance of the work itself which apparently play a determining part. These are load and speed.

Although it might be presumed that load would exercise a marked influence, such experimental work as exists tends to show that increase of load *within limits* does not materially influence the efficiency of the body. There is, however, a slight tendency for the work to be done more efficiently when the load is changed from a moderately heavy to a heavier one than when the change is from a light to a heavy load.

The influence of speed, that is, the rate at which the work is done in unit time, is of much greater moment. Experimentally it has been found that the total energy expenditure per revolution of the pedals is constant for all speeds, but that although there is

naturally an increase in the amount of the total work done, the effective muscular work per revolution decreases as the speed increases, and there is therefore a steady fall in efficiency (see Fig. 101). The same result is obtained when the speed is varied, with, however, approximately the same production of effective muscular work in the two experiments, as shown in Table XC. :

TABLE XC

Revs. per min.	Heat equivalent of external muscular work per min. in Cals.	Net efficiency.*
		per cent.
90	1·94	22·6
124	1·96	15·7
80	1·77	22·1
105	1·83	17·7
71	1·57	24·5
108	1·58	15·6
71	1·34	23·1
94	1·29	20·4
105	1·35	17·0
72	1·20	21·5
88	1·26	19·5

* Base line—complete rest, lying.

As regards the work which has been carried out on isolated muscle, the results which have been obtained are of great interest, as they have led to fresh consideration of the nature of the muscular machine. A. V. Hill, in a long series of ingenious and striking experiments, using special methods of his own devising, has shown that the solution of the problem is not quite so simple as it was formerly imagined. Hill found the simple determination of the mechanical efficiency, *i.e.* W/H, the heat equivalent of the work done, divided by the energy output determined as heat, was of no real importance. The true efficiency of the muscle is the ratio between the " potential energy thrown into an active muscle by excitation " and the " total chemical energy liberated as heat." He found, further, that the heat production varied according to whether the muscle was, or was not, allowed to shorten on stimulation. If shortening were permitted the heat output might be 30 per cent. smaller than if the muscle was

prevented from shortening. On examination of the potential energy developed by a stimulated muscle not allowed to shorten, it was found to be approximately $1/6\ Tl$, where T = the maximum tension and l = the length of the muscle. Hill maintains that the true mechanical efficiency can be determined by comparing this quantity with the heat production. This value $1/6\ Tl$ when expressed in heat units is $10^{-4}/4{\cdot}26$ calories. (See Table XCI.) He found efficiencies approximating 90 per cent. in the initial phases of contraction, and if the whole process, *i.e.* initial and recovery phases taken together, were assessed, the efficiency, under the conditions of his experiments, was in round figures 50 per cent.

TABLE XCI

EXPT.—Length of muscles, 3·3 cm.; weight of muscles, 0·135 gm.; 1 scale division of deflection = $8{\cdot}32 \times 10^{-6}$ cal. Sartorius and isometric contractions.

Duration of excitation: secs.	0·075	0·075	0·075
Initial tension: grm. wt.	10·5	10·5	10·5
Heat production H: cal. $\times 10^{-6}$	574	740	757
Tension T: grms. wt.	44·8	47	47
$Tl/6H$	1·01	0·82	0·80

Incidentally he found that different types of muscle (*e.g.* semimembranosus and sartorius) definitely differed in efficiency. He also found that the maximum efficiency was only obtained under very special conditions of initial tension, strength of stimulus and the physiological state of the muscle.

PART II

ILLUSTRATIVE EXPERIMENTS

"Science has but one language, that of quantity, and but one argument, that of experiment." STARLING.

"The laboratory is the fore-court of the temple of philosophy; and whoso has not offered sacrifices and undergone purification there, has little chance of admission into the sanctuary." HUXLEY.

List of Experiments

Those marked * are suitable for demonstration or for more advanced students.

CHAPTER III.— *1. **Bomb calorimeter.**

CHAPTER V.— 2. **Diffusion.** Gaseous.

3. **Diffusion.** Liquid.

4. **Partial separation of two solutions by diffusion.**

5. **Osmotic pressure** of crystalloids.
   (*a*) By osmometer.
   *(*b*) By determination of the depression of the freezing-point.
   *(*c*) By Barger's method.

6. **Turgor.**

CHAPTER VI.— 7. **Soap films.**

8. **Camel's hair brush experiment.**

9. **Cohesion**—a surface tension phenomenon.

10. **Work done by altering surface tension.**

11. **Effect of soap formation on surface tension.**

12. **Camphor, "Water-beetle"**

13. **Camphor-benzene "amœba."**

14. **Mercury "amœba."**

15. **Electrical alteration of surface tension.**

16. *Ostwald's* **"Physical heart."**

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   (2) Capillary rise method.

18. **Adsorption to a surface.**
   (1) Charcoal and crystalloids.
   (2) Hay's test for bile salts.
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*19. **Demonstration of the oxidation of oxalic acid when adsorbed to charcoal.**

20. **Effect of capillary active substances on the rate of sedimentation of suspensions.**

*21. **Capillary electrometer.**

CHAPTER VII.—22. **Strength of acids.**
   (*a*) By taste.
   (*b*) By hydrolysing power.

(c) By indicators.
*(d) By conductivity.
*23. H ion concentration by potentiometer.
*24. *p*H and surface tension.
25. **Buffer solutions.**
(a) *Sørensen's* (range *p*H 4·5–9·2).
*(b) *Clark and Lubs.* (I.) *p*H 2·2–3·9.
(II.) 4–6·3.
(III.) 5·8–8·0.
(IV.) 7·8–10·0.

CHAPTER VIII.—26. **Separation of colloids from crystalloids.**
(1) Preparation of dialysers.
(2) (a) Albumen + sodium chloride, etc.
(b) Congo red and hydrochloric acid.
(c) Blood serum.
(d) Colloidal iron and hydrochloric acid.

Optical properties of colloids.
*27. *A.* Faraday-Tyndall phenomenon.
B. Polarisation of the Tyndall cone.
*28. *A.* Ultramicroscope.
B. Brownian movement.
29. **Diffusion.**
(a) Colloid into colloid.
(b) Crystalloid into colloid.
(c) Acid into colloid.
(d) Electrical diffusion.
30. **Liesegang phenomenon.**
*(a) Plate rings.
(b) Tube strata.
(c) Effect of capillary active substances on the formation of strata.
(d) Dead space.
*(e) In air.
31. **Viscosity.**
(a) Compare solution, sol and gel.
(b) Effect of mechanical agitation on the viscosity of gelatin.
(c) Effect of concentration on the viscosity of colloids.
(d) Effect of temperature on the viscosity of gelatin.
(e) Effect of electrolytes on the viscosity of gelatin.
(f) Effect of *p*H on the viscosity of acacia.
32. **Determination of the isoelectric point of a protein.**
(a) Casein by acid precipitation.
(b) Gelatin (Loeb's method).
(c) Gelatin by alcohol precipitation.
*33. **Osmotic pressure of gelatin.**
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36. **Coagulation of sols at isoelectric point.**
(a) By heat.
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37. **Protection from precipitation of hydrophobic suspensoids by hydrophilic emulsoids.**
 (*a*) Colloidal iron and gelatin.
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 *(*c*) Lange's test.

38. **Adsorption.**
 (*a*) Colloid to surface.
 (*b*) Colloid to colloid.
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 (*a*) Plates of gelatin or glue and water.
 (*b*) Rubber and benzene.
 *(*c*) Effect of the dielectric value of the imbibed fluid on the amount of swelling.
 (*d*) Pressure of imbibition.
  (i.) Laminaria.
  *(ii.) Gelatin by œdometer.
 (*e*) Heat of imbibition.
 (*f*) Effect of electrolytes on imbibition.
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 (*a*) Reversible and irreversible.
 (*b*) Effect of solutes on gelation.
 (*c*) Effect of non-electrolytes on gelation
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 (*b*) Optimum concentration of stabiliser.
 (*c*) Optimum pH of stabilising colloid.
 *(*d*) Rigidity and concentration of oil.
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43. **Foams.**
 (*a*) Conditions necessary for their formation.
 (*b*) Breaking of foams.
 *(*c*) Adsorption of enzymes by froth.

CHAPTER X.— 44. **Enzymes. General conditions governing enzyme action.**

45. **The influence of pH on enzyme action.**

*46. **The effect of the removal of the end products of enzyme action on the end point of the reaction.**

*47. **Estimation of the relative activity of an enzyme.**

48. **Demonstration of the presence of a lipase in a tissue extract.**

*49. **Estimation of the relative lipolytic activity of an extract of pancreas.**

CHAPTER XI.— 50. **Preparation of semi-permeable membranes. *Chemical "gardens."***

51. **Leduc's "growths."**
*52. **"Shell" formation.**
CHAPTER XII.— 53. **Study of living cells.**
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(*b*) Examination of blood corpuscles.
54. Conditions affecting growth, etc.
*55. Mimicry of cell structure.
CHAPTER XIII.—*56. **Action of ultra-violet light.**
(*a*) On fluorescent substances.
(*b*) Bleaching effect.
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(*d*) On enzymes.
(*e*) On *cyclops.*
(*f*) On human skin.
CHAPTER XIV.—*57. **Indicators of potential difference.**
*58. Current of action and current of injury.
*59. Membrane potential of the skin of an apple.
CHAPTER XV.— *60. Model of mucoid secretion.
CHAPTER XVI.—*61. Model to illustrate some phases of urine formation.

BLOOD

CHAPTER XXII.—62. Specific gravity of blood.
63. Hæmolysis.
64. Fragility of erythrocytes.
65. Viscosity of blood.
66. Clotting time of blood.
67. Bleeding time.
*68. Hæmatocrite.
69. Effect of CO_2 on a buffered solution.
70. Alkali reserve.
(*a*) Approximate.
*(*b*) C. J. Martin's method.
*(*c*) Van Slyke's method.
*(*d*) Van Slyke's micro method.
71. Blood pressure model.
SECTION IV.— 72. Vowel sounds by percussion.
73. Percussion of bladders.
*74. Effect of colour on the absorption of heat.
*75. Use of the kata-thermometer.

PREPARATIONS

76. Distilled water for the Faraday-Tyndall phenomenon and for the ultramicroscope.
77. Collodions.
78. Collodion membranes, etc.
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Typical colloids.

(*a*) **Sols.**

80. Gold.
(*a*) Partially protected.
(*b*) Unprotected.
(*c*) Determination of C_H of colloidal gold.

81. Iron.
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84. Gelatin, 1 per cent.
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86. Gum mastic.
87. Silicic acid.
88. Coarse suspensions.
89. (i.) Egg albumin from eggs.
    (ii.) Egg albumin from commercial egg albumin.
90. Finely divided suspensions of protein for use in experiments on proteases.

(*b*) **Gels.**

91. Egg albumin.
92. Gelatin.

93. Preparation of non-polarisable electrodes.
94. Graphic conversion of Sørensen's pH into concentrations of H ions.
95. Estimation of the surface area of the body.

VARIOUS CONVERSION FACTORS

Graphic conversion of Sørensen's pH into concentrations of hydrogen ions, and the reverse (Roaf).
Estimation of the surface area of the body.
Conversion factors.
A list of some practical handbooks.

1. Bomb Calorimeter.

Measurement of E.V. of Foods by Calorimetric Combustion.—The principle underlying this method is the combustion of a known amount of the material in an apparatus so devised that practically all the heat evolved is absorbed by a known amount of water and by the apparatus itself (which is of known heat capacity). Some form of bomb calorimeter is now universally employed for this purpose. The instrument (Fig. 3, p. 24) consists of three main parts.

1. The bomb itself (Fig. 102) is constructed of steel, nickel-plated, with a cover to be screwed on firmly against a lead washer. It is lined with a special enamel to resist corrosion. Its capacity is about 400 c.c. Through the cover the entrance and exit gas channels pass ; $K2$ with its continuation platinum tube, R, is for the introduction of oxygen, and $K1$ for the withdrawal of the gaseous products of combustion. Both channels are closed by means of the screw spindles $V1$ and $V2$, running in stuffing boxes. $S1$ and $S2$ are screws to stop the lateral communication with $K1$ and $K2$. Through the centre of the cover passes a strong platinum wire, D, and this, as well as R, is fitted with short pegs, a^1, a^2, on which hangs the crucible T. A short collar, just above these pegs, is for the attachment of the ignition wire. $P1$ and $P2$ are two small screw-clamps for attaching to the electric wires for ignition.

2. The insulating chamber is a double-walled copper vessel of about 11 litres capacity, and the space between the walls is to be filled with water at room temperature. It is lined with white enamel, and contains within it, but insulated from it by a thin ebonite stand, 3, the water holder or calorimeter vessel.

3. THE CALORIMETER VESSEL is a cylindrical copper can heavily nickel plated and capable of containing the bomb and about 2,000–2,500 c.c. of water. On the floor of the can is a pad of cork or fibre, on which the bomb rests.

Besides this it is necessary to have a stirring device, a thermometer to read to 1/100° C., and a means whereby oxygen at 15–20 atmospheres pressure can be put into the bomb.

CALIBRATION.—Certain values have to be determined before the apparatus can be employed.

(1) CALORIE VALUE OF MATCH.—In order to convert energy from the potential to the free state, we have already seen that some free energy must be added—the material must be ignited. Various forms of match are employed. Some workers prefer to suspend a dried cotton thread of known weight from a platinum wire connecting D and R. The thread dips into the crucible T, and touches or is embedded in the material to be burned. On completion of an electric circuit through $P1$ and $P2$ the platinum wire glows and sets off the cellulose match, which in turn causes the foodstuff to ignite. Others prefer to weigh out a piece of iron wire, 5–6 cm. long and 0·1 mm. thick, and put it in place of both the platinum wire and the cotton thread. In any case, the amount of heat evolved in the ignition process has to be determined carefully, and deducted from the heat evolved, in a complete estimation or incorporated in the correction called the water equivalent.

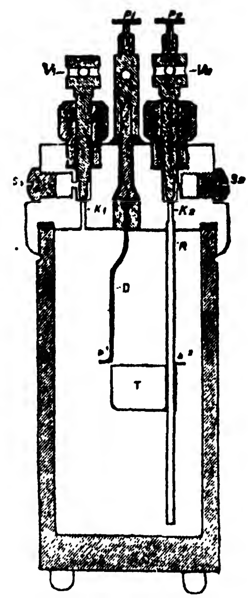


FIG. 102.—Section through a Kroeker bomb (see text).

(2) WATER EQUIVALENT.—The apparatus itself—vessels, thermometer, stirrer—is heated along with the water it contains. Its water equivalent, *i.e.* the quantity of water which has the same heat capacity as the apparatus, must be determined and added to the quantity of water actually employed in the experiment. Several methods exist for this determination. The most exact, and at the same time the most convenient, is to burn in the calorimeter a weighed quantity of a substance whose calorie value is known with absolute certainty, and ascertain the resultant change in the temperature of the water.

If we burn a certain quantity of naphthalene (9,668 calories) or of cane sugar (3,988 calories per gram), which would evolve Q gram cals., the actual rise of temperature shown by the thermometer is $t°$ C., then $Q = (m + \mu)t$ where m = water equivalent of the apparatus and μ = weight of water (in grams) in the apparatus. Transposing, we have

$$m = \frac{Q}{t} - \mu.$$

That is, the water equivalent is :

$$\left(\frac{\text{Total heat generated (calculated)}}{\text{Observed increase in temperature of calorimeter water}}\right) - \left(\begin{array}{c}\text{Quantity of water in}\\ \text{apparatus (in gms.)}\end{array}\right).$$

(3) CALIBRATION OF THERMOMETER.—The thermometer has to be calibrated, and a correction applied for this.

(4) COOLING CONSTANT.—Another correction to be made in the final calcula-

tion is that of the cooling constant of the apparatus. The chief source of error in calorimetric experiments lies in heat exchange with external objects by conduction and radiation. To reduce this error to a minimum (*a*) the chemical action must go on as fast as possible, hence the use of oxygen under pressure; (*b*) the temperature of the calorimeter water is kept as nearly as possible at the same value as the temperature of the room. We have already stated how the grosser errors of conduction and radiation are avoided in the structure of the insulating chamber. In spite of this there is a certain loss, which is measured as part of the regular routine of an experiment and is allowed for.

In order to calculate the energy of any material we must know what the end-products of the combustion are. We have seen that C and H are always under these circumstances completely oxidised to CO_2 and H_2O, which undergo little or no further energy changes. N and S, on the other hand, are converted into sulphuric nitric oxides, which in turn dissolve in and combine with water forming H_2SO_4 and HNO_3. A correction has to be applied for their heats of solution and combination. For very fine work, corrections may be applied for the latent heat of evaporation of water and for the heat of solution of CO_2.

Preparation of the Bomb. Unscrew the cover of the bomb and remove the small bottle or other vessel containing soda lime (which is left in the bomb after each experiment for absorbing moisture). Press the material under investigation into cylindrical shape; weigh accurately (0·5–1 gram) and place it in the quartz (or platinum) crucible (Fig. 102). Fix the crucible T firmly in place with the two little arms of the holder, a^1 and a^2, passing through the two holes in its side. Attach a piece of the ignition wire (*q.v*) to the conducting rods so that it passes straight across and above the crucible. To the centre of this wire, attach the fuse of cotton thread (*q.v.*). The other end of this thread may be incorporated in the substance whose calorie value is being estimated. Examine the lead washer in the cover to ensure the absence of any grit or burr which would interfere with its function as a seal.

After these arrangements are completed, place the bomb in the cast-iron holder (as in *A*, Fig. 3, p. 24), and put the cover on as far as possible by hand. Finish the process with the spanner *C* provided for this purpose. Note that the part of the spanner coming in contact with the bomb is furnished with card gaskets to prevent damage to the plating.

By means of the cone and nut *G* connect the bomb through the pressure gauge and union to a cylinder of compressed oxygen. (i.) Open the inlet valve V_2 (Fig. 102) of the bomb, (ii.) close the fine adjustment release valve (needle valve) on the cylinder, and then (iii.) open the cylinder (main) valve. *Gradually* open the release valve of the cylinder. If this valve is opened too rapidly, the inrush of oxygen will tend to scatter the contents of the crucible.

When the gauge indicates a pressure of 15–20 atmospheres, close (i.) release valve, (ii.) inlet valve of bomb, and (iii.) cylinder main valve. Disconnect and place bomb in inner calorimeter vessel.

Preparation of Calorimeter. The calorimeter—a heavily nickel-plated polished cylinder of copper should have placed in it about 2,000 *grams* of water (accurately weighed). The calorimeter vessel and its charge of water are best kept in an outer room with a temperature about 1° C. below that of the room in which the calorimetric combustion is to be done. When the bomb is placed in it, the water should cover the oblong excrescence, but not the terminals P_1 and P_2 nor the valve spindles V_1 and V_2. One can now see whether the bomb is gas tight or not. Small leaks do not render the experi-

ment useless, but they are best avoided. Leave for half an hour. Place the calorimeter, carefully carried in a cloth, on the cork studs on the thin sheet of ebonite on the bottom of the large double-walled insulating vessel *H* (Fig. 3), which has been filled with about 11 litres of water. Connect the terminals P_1 and P_2 by well-insulated flexible leads to a battery (4–6 volts) provided with a simple contact key. Place the ebonite cover, stirring gear and thermometer in position.

Start the stirring device (best activated by a small electric motor) and after the lapse of a few minutes take readings of the thermometer regularly every half-minute. As the water in the calorimeter is about 1° C. below the temperature of the room, the readings will tend to rise, *e.g.*

I. PRE-IGNITION PERIOD.

Time (minutes)	0	½	1	1½	2
Temp. (° C.)	14·953	0·955	0·957	0·958	0·960
Time (minutes)	2½	3	3½	4	
Temp. (° C.)	0·963	0·964	0·966	0·969	

i.e. *rate of warming at* 14·9° *C. is* 0·002° *C. per half minute.*

Ignition. At a known moment, fire the charge by pressing the button at the battery. The temperature rapidly rises. Take readings every half-minute as under.

II. IGNITION PERIOD.

Time (minutes) . .	4½	5	5½	6	6½	7
Temp. ° C. (14 omitted) .	1·5	2·1	2·3	2·4	2·45	2·47
Time (minutes) . .	7½	8	8½	9	9½	10
Temp. ° C. (14 omitted) .	2·496	2·503	2·508	2·510	2·512	2·511

i.e. the temperature reaches a maximum value six minutes after ignition and then begins to fall. Readings are continued every half-minute till the fall of temperature has become quite regular, *e.g.*

III. COOLING PERIOD.

Time (minutes) . .	10½	11	11½	12	12½	13
Temp ° C. (14 omitted) .	2·51	2·508	2·507	2·505	2·504	2·502
Time (minutes) . .	13½	14	14½	15	15½	16
Temp. ° C. (14 omitted) .	2·5	2·498	2·496	2·493	2·490	2·488

i.e. the rate of cooling during this period is 0·002° C. per half-minute.

From these two cooling values, *e.g.* 0·002° in the pre-ignition period and

$+ 0{\cdot}002°$ in the cooling period a cooling curve may be constructed with the temperatures from 0·9 to 2·9 as abscissæ and cooling loss from $- 0{\cdot}002$ to $+ 0{\cdot}002$ as ordinates. The function is purely linear and extrapolation is simple.

Calculation of E.V. (a) *Correction of Temperature.* From the cooling curve it is easy to discover the loss of heat due to cooling in the ignition period. This value should be placed in a third column for every temperature recorded during the second and third periods. For any reading of the thermometer, the total loss by radiation is obtained by summing up the losses in all the preceding intervals after ignition, *e.g.* in the second period the losses were $- 0{\cdot}001$, $+ 0{\cdot}001$, 0·0015, 0·0016, 0·0018, 0·0018, 0·0019, 0·002, 0·002, 0·002, 0·002, 0·002. This gives a total loss of 0·0175 at the maximum temperature observed. This value added to the observed temperature gives the corrected temperature. The corrected temperature during the third period varies so little that the arithmetic mean of the values is taken as the corrected maximum temperature. In this case = 16·5325.

(b) *Calculation.*

Temperature before firing, 14·969.
Temperature after firing (corr.), 16·5325.
∴ Rise in temperature, 1·5635.
Water in calorimeter = 2,100 gram.
Water equivalent of calorimeter = 767·5 gram.
∴ Total water value = 2867·5 gram.
Heat of combustion = 2867·5 × 1·5635 = 4484.
Amount of N free food burned = 1·2 gram.
∴ EV of food = 4484/1·2 gram.
= 3737 cals. per gram.
= 3·73 Cals. per gram.

As soon as possible after each estimation, the gases are let off and the bomb opened. Wash out the bomb with dilute sodium hydrate to remove any nitric acid formed, dry and replace the bottle containing soda lime. Close the bomb.

2. Gaseous Diffusion.

Experiment on p. 38, Fig. 4. Try this first with coal-gas and then with CO_2. Soak the porous pot in water and compare the rate of diffusion inwards of carbon-dioxide with the outwards diffusion of air. What part does solubility play in diffusion through a membrane ?

3. Liquid Diffusion.

Place a number of coloured solutions varying in nature and in concentration in test-tubes. Carefully fill the tubes with distilled water and note the rate at which the colour diffuses upwards into the water (Fig. 5 (a), p. 39).

(*a*) *Nature.* Use concentrated solutions of copper sulphate, potassium bichromate, methylene blue, congo red, black Indian ink, etc.

(*b*) *Concentration.* Take four test tubes and put 5 c.c. of distilled water in each. To the first tube add 5 c.c. of conc. $CuSO_4$ and mix thoroughly. Remove 5 c.c. of this mixture and add it to tube 2. Mix and take 5 c.c. for tube 3 and so on, rejecting 5 c.c. of the mixture in tube 5. This will give you five samples of 5 c.c. each varying in concentration from tube 1, having 0·5 conc.; tube 2 = 0·25, tube 3 = 0·125 and tube 4 = 0·0625. In tube 5 place 5 c.c. of the conc. solution. Now carefully fill the tubes with water so as to form a clear layer of water above the blue sulphate and measure the rate

of diffusion. A narrow strip of translucent squared paper pasted over the length of the test tube will aid in the determination.

(*c*) *Temperature.* Use two samples of conc. $CuSO_4$—one at 0° C. and the other at 40° C.

Note that the water added should be at the same temperature as the solution and the tubes should be kept at constant temperature during the duration of the experiment.

4. Partial Separation of two Solutions by Diffusion.

(i.) Add sufficient dilute alkaline aqueous *eosin* to a solution of *night blue* to give a dark violet mixture. Allow this mixture to stand in contact with water (as above) for a day. The supernatant fluid will be stained red and the underlying fluid will be a bluish violet.

(ii.) Make some *congo red* just blue by the addition of a few c.c. of N/10 sulphuric acid and allow this blue liquid to lie in contact with water tinged with *phenol red* for twenty-four hours. The acid diffuses from the congo red into the water. The result is a yellow fluid lying over a red one.

(iii.) Other pairs of rapidly diffusible and slowly diffusible substances are: picric acid + alkali blue, picric acid + alizarin red, alkali blue + acid fuchsin.

5. Osmotic Pressure of Crystalloids.

(*a*) **By Osmometer.** *Preparation of Semipermeable Membrane.* Take a clean porous pot such as is sold for Leclanché units. Allow it to soak for a day in distilled water. Fill it with a 0·25 per cent. solution of copper sulphate and immerse it in a 0·21 per cent. solution of potassium ferrocyanide for a day or two. Wash thoroughly in distilled water. The copper sulphate and potassium ferrocyanide meet in the porous pot and a membrane of copper ferrocyanide is there formed (see Expt. 50). The prepared pot may keep for years and be used many times.

A rubber stopper with two holes should be permanently fixed in its mouth with wax. Through one hole should be passed a long glass tube or a U-shaped glass manometer. The other hole carries a tap funnel for filling the pot. The solution to be tested should be coloured with methylene blue or other dye which is easily seen.

(i.) What happens after 24 hours or so when a 10 per cent. cane sugar solution is placed in the pot and the pot immersed in water?

(ii.) Now add sugar to the fluid outside the pot till its concentration is the same as that inside the pot and leave for the same period as before.

(iii.) Increase the concentration of sugar outside and note the effect on the level of fluid in the manometer.

(iv.) Clean out the pot and fill it, in turn, with the following solutions:—

$M/64$ cane sugar.
$M/128$ sodium chloride.
$M/192$ calcium chloride.
$M/192$ sodium sulphate.

They should all rise to the same height in the same time, *i.e.* they are isotonic solutions.

(v.) Prepare an osmometer with a collodion membrane (as in Expt. 33) and again determine the relative osmotic pressures of the above four solutions. The rise in hydrostatic pressure, in the case of a collodion membrane, is not equal for solutions of equal osmotic pressure. The cane sugar in half an hour shows scarcely any osmotic pressure, the $CaCl_2$ solution gives the greatest rise, about 50–60 mm., next comes the NaCl with about 15 mm., and the Na_2SO_4 at from + 5 to − 3 mm. Why do these values differ

from those obtained in Expt. (iv.) above? Why should a negative pressure be found in some cases?

(vi.) Fill the collodion osmometer with distilled water and immerse it in $N/1000$ HCl. The water rises in the pressure tube. Why is this so?

(*b*) **Determination of the Freezing-point of Urine. Principle.** The freezing-point of water is depressed by the addition of salts which go into true solution. The magnitude of the depression (termed Δ) bears a relation to the molecular concentration of the solutes and therefore to their osmotic pressure.

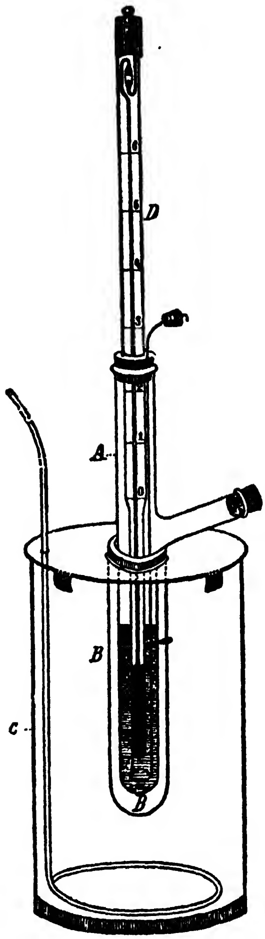


FIG. 103.—Freezing-point Apparatus.

Apparatus. Beckmann's (Fig. 103). It consists of a specially devised test tube *A* with a side neck. Through the rubber stopper, closing the main neck of this tube, pass a thermometer *D* and a short glass tubular guide for a stirrer. The freezing-point tube is supported in the neck of a large test tube *B*, by means of a cork or asbestos ring so that the freezing-point tube is protected from incoming heat by a mantle of still air. This ensures that the cooling of the liquid in the freezing-point tube is slow and fairly uniform. The whole apparatus is inserted through a hole in the middle of a brass sheet, to which it is fixed by a ring of cork or of asbestos. The sheet of brass acts as a lid to a glass jar *C* which contains powdered ice and salt—the cooling bath. Other holes in the lid permit of the passage of a stirrer, a thermometer, and a test tube containing pure water.

The Beckmann Thermometer. The thermometer in the freezing-point tube must be graduated to, at least, hundredths of a degree. Such a thermometer, if made in the ordinary way, unless it were inconveniently long, would have a very short range. To obviate the necessity of having a series of thermometers for use over various ranges of temperature, Beckmann designed one which may be set to indicate temperatures over any desired range. This result is produced by a device permitting of alterations being made in the amount of mercury in the bulb. At the upper end of the thermometer (Fig. 104) there is a small reservoir into which the excess of mercury may be driven, or from which a larger supply of mercury may be obtained.

Setting the Beckmann Thermometer. Hang the thermometer in a beaker of water, the temperature of which is 2–3° higher than the highest temperature to be met with in the experiment and see whether or not the top of the mercury comes within the scale.

A. If there is too much mercury in the bulb and the column rises beyond the graduated part, the excess is removed by warming the mercury in the bulb till the column of mercury unites with the mercury in the reservoir. This is done, (*a*) by placing the bulb in water just a little warmer than before. (*b*) When the mercury passes to the top of the capillary tube and

forms a small drop there, the thermometer should be carefully inverted and tapped gently so as to cause the mercury in the reservoir to coalesce with the mercury in the top of the capillary. (*c*) The thermometer is returned to the upright position by a gentle steady movement and its upper end is struck a sharp tap against the palm of the hand, causing the excess of mercury to break off from the end of the capillary. The thermometer is again tested in the first bath.

B. If, on the other hand, the amount of mercury in the bulb is so small that the top of the column does not rise to the top of the scale, more mercury will have to be drawn from the reservoir. The procedure is similar to that outlined above, but at (*c*) the thermometer is replaced in the first bath before breaking the mercury column. That is, the mercury in the bulb is allowed to contract and draw in more mercury from the reservoir before the connection between column and reservoir is broken by tapping.

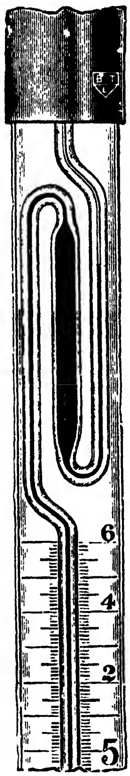


FIG. 104. Upper portion of the stem of a Beckmann Thermometer, showing mercury reservoir.

These operations are repeated till the proper level of mercury has been attained. *This is always tested by placing the thermometer in baths having temperatures equal to the highest and lowest to be encountered in the experiment, and noting that the top of the column of mercury remains on the scale.*

Method. (1) Set up the apparatus completely so as to ensure all parts fitting properly. See that the stirrer in the inner tube is working smoothly and does not strike against the bulb of the thermometer.

(2) Remove the thermometer and stirrer from the tube. Clean and dry the latter.

(3) Pipette in 25 c.c. of urine.

(4) Set the Beckmann thermometer so that, at 0° C., the mercury stands not lower than the middle of the scale.

(5) Dry the thermometer and insert it along with the stirrer in the freezing-point tube, so that the bulb of the thermometer is completely immersed in the urine.

(6) Fill the outer cooling vessel with water, ice and salt. The freezing-point of urine can now be determined.

(7) First make an approximate determination by placing the freezing-point tube directly in the cooling bath so that a rapid fall of temperature occurs.

(8) As soon as the urine shows signs of freezing remove the tube from the freezing mixture, dry it quickly and place it in the air jacket in the cooling bath.

(9) Stir slowly and read the temperature when it becomes constant.

(10) Withdraw the tube and melt ice by warming with the hand, trying to avoid raising the temperature more than 1° C.

(11) Rapidly dry the tube and reinsert it in the air jacket and repeat the freezing process, stirring *slowly all the time.*

(12) When the temperature has fallen to from 0·2° to 0·5° below the approximate freezing-point found in (9) stir more vigorously. This generally is sufficient to induce solidification to commence and the temperature will now begin to rise.

(13) If so, stir slowly and take readings of the temperature every few seconds—tapping the thermometer each time before reading. Note the highest temperature reached.

(14) Again melt and repeat the determination. At least three determinations of the freezing-point should be made, the mean being taken. The deviations of the chosen readings from the mean should be less than 0·002° C.

(15) The depression of the freezing-point or, in this case, the thermometric readings may be converted into osmotic pressure in metres of water by multiplying by the factor 122·7.

Thus suppose that the Δ observed is $-$ 2·3°, the osmotic pressure of this sample of urine would be 2·3 $\times$ 122·7 = 282·2 metres of water = 282·2/13·6 = 20·7 mm. of mercury.

Precautions. (α) The temperature of the cooling bath must not be too low. It should not exceed 3° below the freezing-point of the liquid.

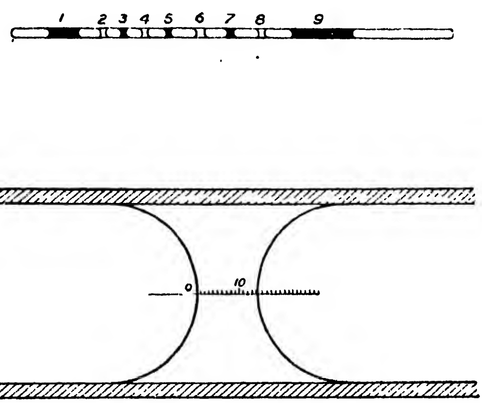


FIG. 105. Barger's Method for Determining Molecular Concentration.
Upper figure, actual size. Lower figure, as seen under the microscope; micrometer scale in eyepiece.

(β) Excessive supercooling should be avoided. It should not be greater than half a degree.

(γ) Stirring should not be too rapid—say one up-and-down movement per second, and it should be as uniform as possible.

(δ) If the liquid shows a tendency to give up heat without freezing and that even vigorous stirring does not initiate solidification, the introduction of a small crystal of ice through the side tube generally suffices to start solidification.

(*c*) **Osmotic Pressure by Barger's Method** (Fig. 105). *Trans. Chem. Soc.* 85, p. 286. Prepare a number of capillary tubes by drawing out soft glass tubing of ½ in. bore into capillaries 1–2 ft. long. These should be cut into smaller pieces, having a smooth regular edge, in order that the tube may be closed tightly with the finger while it is being filled. The internal diameter of the capillaries should be between 1 and 2 mm., preferably about 1·5 mm.

The filling of the tubes requires a little practice. The tube is taken between the middle finger and thumb, and its upper end, which should be rounded, is closed with the index finger. The other end is then dipped below the surface of solution *A*. By lifting the index finger very slightly

enough liquid is admitted into the tube to make a column of about 5 mm. long. The finger is replaced on the end of the tube, which is then lifted from the fluid and inverted so that the open end is uppermost. It is held in a slanting position, and, by diminishing the pressure of the index finger on the lower end, the globule of liquid is allowed to slide down the tube, its progress being regulated by the slant of the tube. The process is repeated, using solution B and so on, using solution A and B alternately and finishing with A. When all the drops are in, the collection is moved so that the last drop is about 1 cm. from the open end of the tube, and this end is sealed in a *small* bunsen flame. The other end of the tube may then be similarly sealed. The upper diagram, in Fig. 105, shows the appearance (actual size) of a filled and sealed tube. The dark drops are A. The first and last drops, 1 and 9, are large and are not taken into account. The drops are numbered in the order in which they were put into the tube, *i.e.* the open end of the tube is to the right of the diagram. The tubes are cemented to a microscope slide and a coverslip fixed with Canada balsam. The slide is placed in a Petri dish with enough water to cover the tubes. (Constant temperature.) Under the microscope the tubes present an appearance like that shown in Fig. 105, lower diagram. With an eyepiece micrometer, measure the drops. After an interval whose length depends on the solvent, the drops are remeasured.

In what direction, if any, does the alteration in size take place? That is, do the drops of B become larger or smaller? If B drops increase in size it shows that the vapour pressure of B is less than that of A, and, consequently, the osmotic pressure of B is greater than that of A, and *vice versâ*. A = 2·5 per cent. glucose in water, B = 2·5 per cent. NaCl in water. Time about 20 hours.

6. Turgor.

(See Expts. 26 and 79 for precautions). Take a length of sausage-skin parchment. Close one end tightly round a glass stopper. Fill with treacle or a strong solution of sugar and then similarly close the other end. Suspend horizontally in water from a loop round the middle. The ends, which droop at first, giving the whole the appearance of an arch, soon begin to assume a horizontal position. In a day or so the sausage skin will be rigid and straight (Fig. 45).

7-21. Experiments on Surface Tension.

Soap Solution. In performing these experiments it is necessary to have a good soap solution. It may be made as follows from pure sodium oleate and glycerol. To 600 c.c. of distilled water in a stoppered bottle of 1 litre capacity add 15 grams of pure sodium oleate (in flakes), and by occasional shaking *in the cold* get it into solution. This may take a day or two. Then add 200 c.c. of pure glycerol, shake and allow to stand, undisturbed in the dark, for a week. Siphon off the clear *underlying* fluid and add 4 drops of concentrated ammonia. Keep well stoppered and away from light.

7. Experiment with Soap Films.

Make a film on the wide end of a conical tube (filter funnel), closing the other end with the finger. What happens when the finger is removed? Where does the film come to rest and why?

8. Camel-hair Brush Experiments.

Many illuminating experiments may be made with a small camel-hair paint pencil. Under water the hairs diverge, but when the surface tension of the

water-hair surface is increased, *e.g.* by removing the brush from the water, the hairs form a compact pencil.

9. **Boy's Leather Sucker** (p. 392).

To show that surface tension is the causative factor suspend a microscope slide horizontally in the receiver of an air pump. By means of a drop of water between, cause a second slide to adhere to the lower surface of the first slide in such a way that the second slide may be loaded. Load with the maximum weight and exhaust the receiver. Repeat the experiment under various conditions, *e.g.* trace of oil ester, bile salts, etc.

10. **Work Done by Altering Surface Tension.**

In performing the experiment detailed on p. 175 (Fig. 42), it is convenient to have a clean copper frame, easily prepared by bending a piece of copper wire (No. 16 gauge) into a circle about 2 inches in diameter, with a handle about 2 inches long. The frame is freed from grease by washing in dilute soda and then thoroughly rinsed. A piece of silk thread is attached to one portion of the frame and a loop made on its loose end. Some of the soap solution is placed in a large Petri dish. The frame is dipped into this and removed horizontally with a fine film of soap covering the circle. Satisfy yourself that the loop moves freely over the film by gently rotating the frame. Now break the film inside the loop, using for this purpose a bluntly pointed piece of blotting paper.

11. **Effect of Soap Formation on Surface Tension.**

Take four similar watch-glasses. In the first two put a few c.c. of water, and in the other two about the same quantity of a 1 per cent. solution of sodium carbonate. With a fine pipette place one drop of rancid olive oil on the surface of the liquid in glasses 1 and 3. Place drops of liquid paraffin of similar size on the fluid in glasses 2 and 4. Note (*a*) whether the rancid oil and the paraffin differ in spreading power on a water surface, and (*b*) the movements occurring in glass 3. Can you explain these movments in the light of what you have learned from experiments 9 and 10?

12. **Camphor "Water-beetle."**

Prepare a rectangular piece of camphor. To one short side affix a short piece of stick and place the whole thing on the surface of water in a large dish. How do you explain the direction of the movements? Remove the stick and replace the camphor in the water.

13 **Camphor-Benzene "Amœba."**

(Brailsford Robertson.) The amœba is made of a saturated solution of camphor in benzene to which a dye (*e.g.* carmine) has been added to make the solution easily visible when placed in water. Place a drop of the solution on the surface of *clean* water in a *clean* Petri dish. The movements may be slowed down by the addition of the faintest trace of oil. Generally the first "amœba" disintegrates rapidly. Do not throw out the fluid in the dish, but add another drop of the camphor solution. What effect has increase or decrease in temperature on the movements? What happens when a solid particle is suspended in the water near the "amœba"? What is the effect of putting two separate drops on the surface of the water at the same time, (*a*) when the drops are equal in size, (*b*) when they are unequal in size? What is the effect of the addition of a trace of fat, obtained, for example, by touching the surface of the water with a glass rod which has been rubbed on the side of the nose?

14. **Mercury "Amœba."**

Place a small globule of mercury in a large Petri dish and cover it with

potassium bichromate (sat. solution) to which has been added some nitric acid (bench reagent). How do you explain the movements ?

15. **Electrical Alteration of Surface Tension.**

Carry out the experiment detailed on p. 49. Place a small globule of mercury on an iron or enamelled plate and cover with water. Note shape of globule. Add sulphuric acid (bench reagent) drop by drop (to make about a 10 per cent. solution). What happens to the globule ? Explain. Connect the plate and globule to a single cell of a battery through a commutator. First pass the current through the globule to the plate and note alterations in shape, then reverse the commutator.

16. **Ostwald's Physical "Heart."** (Verworn's *Physiologisches Praktikum.*)

The method of carrying out this demonstration of electrical alterations in surface tension is indicated in the diagram (Fig. 11, p. 49). A globule of mercury about an inch in diameter is placed in a clock glass almost filled with 10–15 per cent. sulphuric acid. Potassium bichromate solution (say $N/10$), is added drop by drop till the fluid becomes a light yellow in colour. A *clean* sewing needle thrust through a cork is placed in a diagonal position so that the point of the needle just touches the margin of the mercury globule. At the moment of contact the globule becomes more spherical. This breaks its contact with the needle and it loses its semispherical form and so again makes contact. These rhythmic pulsations may go on for hours. When the action has stopped remove the needle and note the odour of acetylene. How do you account for this ? What is the reason for adding bichromate ?

17. **Measurement of Surface Tension.**

Simple Stalagmometer. A simple stalagmometer which is sufficiently accurate for any of the experiments detailed here may be made from commercial capillary glass tubing. Cut a piece of this tubing into sections of 7 to 10 cm. long and grind the ends flat on fine emery cloth moistened with paraffin oil. By means of a short length of pressure tubing fix the capillary tube (glass to glass) to the lower end of a 3 to 5 c.c. pipette graduated to 1/10 c.c. and clamp rigidly in a vertical position over a narrow graduated cylinder or other vessel to catch the drops.

Note.—The stalagmometer should be dissociated into its component parts except when actually in use and the glass parts kept in cleaning mixture. Before use they should be thoroughly rinsed with warm distilled water and dried by attachment to a sucking or blowing apparatus.

To Calibrate the Stalagmometer. It is convenient to calibrate the apparatus at room temperature. Fill it to above the top graduation mark with distilled water which has attained room temperature. Start counting and collecting the drops when the meniscus just passes the top mark. Note the graduation mark when the sixtieth drop falls. Repeat the experiment to confirm this point and then by a deep file mark or other means intensify this mark.

To compare the surface tensions of distilled water and 0·1 per cent. soap solutions.

(i.) **Stalagmometer Method.**

(*a*) Confirm the calibration of the stalagmometer by counting (i.) the number of drops, (ii.) the volume of the fluid dropped, and (iii.) the time taken in dropping between the two marks.

(*b*) Wash out the apparatus with the soap solution and then fill to above the top mark with the soap solution and proceed as in (*a*).

TYPICAL RESULTS AT 17° C.

	Water.	Soap Soln.
Liquid to be tested	Water.	Soap Soln.
Number of drops	60	112
Total volume of drops	3 c.c.	3 c.c.
Time taken for fluid to fall from upper to lower mark	43 secs.	50 secs.
Surface tension relative to water	$\frac{60}{112} = 0{\cdot}5$ approx.	

(ii.) **Capillary-Tube Method.** A fluid which wets the tube will rise in it to a height h, which may be calculated from the formula $h = \frac{2\sigma}{rD}$ where $\sigma =$ surface tension, $r =$ radius of the capillary, and $D =$ specific gravity of the liquid. For approximate determinations, the height h may be measured by calipers against a millimetre scale. Short lengths of thermometer tubing may be used, having an internal diameter of from 0·3 to 2 mm. The tubes should be prepared and cleaned as directed in Experiment 17 (i) above.

(*a*) Select a tube, thoroughly rinse it in distilled water and clamp it vertically, dipping into distilled water. Be certain that the meniscus moves freely in the tube, and that no drops of liquid adhere to the internal walls above the meniscus. If this is not so, the tube is dirty and a fresh clean one should be selected.

(*b*) Measure the height to which distilled water (at room temperature) rises in the tube. Repeat, using the soap solution above. See also Experiment 48.

18. **Adsorption to a Surface.**

(1) *Adsorption by Charcoal.* (*a*) Put 10 c.c. of a 0·1 per cent. solution of *crystal violet* + 1 gm. of bone charcoal into a tube. Shake. Filter. The filtrate is colourless. Return the precipitate to the cleaned tube. Add 10 c.c. of distilled water. Shake. Practically no colour leaves the charcoal for the water. Now add a few cubic centimetres of methylated spirit and shake. The dye leaves the charcoal.

(*b*) Repeat the experiment, using N/100HCl in place of the dye. Test the filtrate with *methyl red*. The hydrochloric acid has been adsorbed completely.

(2) *Hay's Test for Bile Salts.* The surface tension of water is sufficient to permit of placing " flowers of sulphur " on it without rupturing the surface.

Take three test tubes and place in (i.) about 10 c.c. of water ; in (ii.) about 5 c.c. of water + 5 c.c. of methylated spirit well mixed together ; and in (iii.) 10 c.c. of water containing bile salts (or ox bile). On to the surface of each tube, sprinkle a small pinch of powdered sulphur. In tubes (ii.) and (iii.) the sulphur will rupture the surface and sink to the bottom of the tube.

(3) (*a*) *Flotation.* Prepare a finely ground mixture of 9 parts of animal charcoal and 1 part of aluminium silicate (clay). Shake 3 or 4 gms. of this mixture with about 100 c.c. of water, so as to get a suspension. Allow to stand and decant off the supernatant liquid. Repeat this process so as to get rid of the coarser particles of the clay.

Now shake the greyish black suspension with any capillary active substance (*q.v.*), and pour the mixture into a clean boiling-tube. The mixture will separate into two sharply defined layers. The charcoal will adhere to the glass-, air- and water-interfaces of the upper liquid (*e.g.* benzol), while the lower aqueous layer will contain practically all the clay.

(*b*) *Flotation.* (Ostwald.) Cut a few pieces from a *sized* paper heavily printed on one side, and the same number of similar size from a similar un-

printed sheet. Shake the pieces of paper in an Erlenmeyer flask containing water till the papers have become waterlogged and sink to the bottom of the flask. Cover the water with a thin layer of a light mineral oil, and shake thoroughly. When the oil and water have separated, the printed bits of paper will be found floating, print side upwards, at the oil-water interface. The unprinted bits sink. Paraffin oil, the usual dispersion medium for printer's ink, is a satisfactory oil to use.

19. Demonstration of the Oxidation of Oxalic Acid when Adsorbed to Charcoal.

Air freed from CO_2 (by passing over soda lime, caustic soda and baryta) is sucked through a vessel containing charcoal, oxalic acid and water. The CO_2 liberated by the oxidation of the oxalic acid is passed into lime or baryta water which becomes turbid.

Arrangement of apparatus. 1 Suction pump, 2 trap, 3 Woulfe's bottle with lime water, 4 Erlenmeyer flask, 5 Woulfe's bottle with lime water, 6 and 7 two gas wash bottles with a layer of 40 per cent. sodium hydroxide, and 8 a soda lime tower. By means of a clip on the inlet tube of the tower, the flow of air is regulated through the series of vessels so that the bubbles in bottle 3 can just be counted.

Method. See that the bottles and flasks are connected in the right way so that the air enters by the long tube dipping into the fluid in the bottle. The reaction vessel (No. 4) is a flask fitted with a rubber stopper carrying three tubes. Of these, two tubes are like those of the other flasks. The third tube goes almost to the bottom of the flask and at its upper end bears a funnel and stop-cock. This flask is kept at about 100° C. by immersion in a bath of boiling water.

First test the apparatus for leaks by running it with the inlet on the tower closed. Now run in the charcoal mixture. (In order to free the charcoal as far as possible from adsorbed CO_2, it is heated dry and allowed to cool in a desiccator over NaOH.) For our purpose, take 5 gms. of Merck's blood charcoal and suspend it in 50 c.c. of boiled water. Boil the suspension for 15 minutes and run it, while still warm, into the already warmed reaction chamber. Wash the final grains into the reaction flask with 50 c.c. of boiling water. Run the pump for a short time to remove the last traces of CO_2 from the charcoal. This may require an hour or so. Now put fresh lime water into bottle 3. Start the pump again. If no turbidity develops in 3, the experiment may be begun. If turbidity does develop, proceed as before.

Experiment. Stop the suction and through the funnel add 2 gms. of oxalic acid (dissolved in the minimum amount of water) to the reaction vessel. Start the pump. In the course of a few minutes the lime water in bottle 3 will be quite milky in appearance. It is advisable to run a blank experiment with oxalic acid and 100 c.c. of water but no charcoal in flask 4.

20. Effect of Capillary-active Substances on the Rate of Sedimentation.

Take three test tubes and put about 20 c.c. of water into each. To one tube add a few grains of camphor; to the second a drop of tributyrin. Shake thoroughly and add to all three about 2 gms. of finely powdered kaolin. Shake vigorously and leave for about an hour. The kaolin slowly sinks to the bottom in all the tubes. The camphor and the tributyrin almost double the rate of sedimentation. Repeat the experiment, using powdered charcoal instead of kaolin. Can you explain why the capillary active substances increase the rate of sedimentation of the kaolin and not of the charcoal? (See Experiment 18.)

21. **The Capillary Electrometer.**

This instrument for measuring *differences of electric potential* depends for its action upon the alteration of surface tension between mercury and sulphuric acid with alterations of the potential difference at the interface (see p. 50, and Experiments 16 and 17). For class use the simplest satisfactory form is that made by the Harvard Apparatus Co. It consists of a capillary tube containing mercury which is continuous with a reservoir in the form of a plunger pump. The position of the mercury in the capillary may be altered by adjusting the plunger by means of a micro-screw. The glass capillary dips into a small test tube containing dilute sulphuric acid and a drop of mercury to make good contact with the platinum wire sealed through the bottom of the tube. This platinum wire and the one coming from the mercury in the capillary are short-circuited through a spring key. The whole instrument is placed on a microscope stage set vertically.

Details. (*a*) Mercury. Pure dry mercury must be used. *To clean mercury:* shake for 10–20 minutes with a solution of mercurous nitrate acidified with nitric acid. Wash thoroughly with distilled water and dry with filter paper.

FIG. 106.—Capillary electrometer.

(*b*) Sulphuric acid. The pure (boiled) acid in six times its volume of distilled water is shaken up with a little pure mercury and is best kept in contact with some mercury.

(*c*) The glass parts must be free from grease and the rubber connections from French chalk.

Filling and Setting the Electrometer. Fill the pump-reservoir with mercury, allowing free access to the capillary. Before inserting the plunger, cover the mercury surface with a film of thin oil (balance oil). The insertion of the plunger will cause mercury to be forced through the capillary. Fix the capillary in position in the test tube, which should be half full of acid. A slight turn of the plunger screw will force a little mercury into the test tube to cover the platinum contact. Adjust by means of the plunger screw till the Hg–H_2SO_4 interface lies in the middle of the microscopic field. The definition of the mercury meniscus may be improved by cementing a cover glass to the test tube with Canada balsam. An eyepiece micrometer provides a scale whereby the movements of the mercury may be measured.

For finer work, the closed type of electrometer (Fig. 106) gives excellent results, and, once adjusted, needs no further attention for months.

Before this piece of apparatus is set up, tilt the tube until the greater part of the mercury has run over from *B* to *T* (Fig. 12). Set the tube with the bulb limb (*B*) sloping slightly downwards and allow mercury to pass along *U* and *A* from *T* and to drip into *B* until, on suddenly righting the tube, the mercury stands midway in *A* as in the figure. Fix in the stand and focus the microscope on the meniscus. Affix the leads *E* and *F* as in the diagram (Fig. 12), taking care to see that the short-circuiting key is across them and is kept closed.

After any capillary electrometer has been readjusted so that a new surface has been formed at the meniscus, it is essential to allow this surface to come into electrical equilibrium with the glass and the sulphuric acid. (See Ageing of Surfaces.) For rough work a few hours' rest is enough, but about 24 hours is a preferable interval. Test the sensitivity of the electrometer by opening the short-circuiting key and noting the movement, if any, of the meniscus.

The meniscus should either remain steady or should move only slightly. To ensure that the lack of movement is not due to lack of sensitivity, pass a weak current momentarily from F to E and note whether the electrometer responds with a movement of the mercury from A towards U. A suitable weak current may be generated by attaching a piece of brass wire to the distal end of P and a copper wire to the corresponding part of F, and, after opening the s.c. key, touching these wires with different fingers. Reverse the wires and note whether a movement of the mercury in the reverse direction is obtained.

To Test the Sensitivity of the Electrometer. Place a 2-volt accumulator which is needing to be recharged (*i.e.* E.M.F. of about 1·85 volts) at Z in the circuit of Fig. 12 with its + pole attached to R and – pole to P. Close the main circuit and open the short-circuiting key for a moment (1 to 2 seconds), observing meanwhile the mercury meniscus. It should move apparently upwards in the microscope (actually downwards). Move the slide-wire Q either towards P or R, and again fully depress the key so completing the electrometer circuit and removing the short between E and F. If the meniscus has a smaller excursion than before, release the key (*i.e.* short the terminals and open the accumulator circuit) and move the slider a little more in the same direction as before. (If the excursion of the mercury, on the other hand, were increased, the slider would have to be moved in the opposite sense.) Continue the process of moving Q, and completing the electrometer circuit till a point of balance between P and R is reached. A sensitive electrometer should show a deflection of 2 to 4 scale divisions in the eye-piece micrometer when the slider is 1 mm. from the point of balance. From this division of the potentiometer wire can be calculated the potential at the mercury surface in the bulb, or if an accumulator of unknown strength is placed at Z and a standard cell (*e.g.* cadmium cell) is placed between P and E to give a negative potential to the mercury in T, one can measure the E.M.F. of the unknown as follows. Since the combined resistances of the leads are negligible compared with the resistance of the potentiometer wire $P\ R$, there exists the same difference of potential between the points P and R as between the terminals of the unknown source of E.M.F., *i.e.* E_{PR} = E.M.F. of the accumulator.

When the point of balance has been obtained with Q, the potential difference between Q and P ($= E_{PQ}$) is equal to the E.M.F. of the cell E(cad).

Now $$E_{PQ} : E_{PR} = \text{Length } PQ : \text{Length } PR,$$

i.e. $$E_{PR} = E_{PQ}\,\frac{PR}{PQ}$$

$\therefore$ $$\text{E.M.F. of accumulator} = E(\text{cad})\,\frac{PR}{PQ}$$

For example, if E(cad) = 1·0185 volts and PR = 1,000 mm. and PQ = 550·5, then $E(\text{acc}) = \dfrac{1{\cdot}0185 \times 1{,}000}{550{\cdot}5} = 1{\cdot}85$ volts.

If the electrometer is used to find the point of balanced E.M.F. on a Wheatstone bridge (Experiment 22 (*d*)), it is essential to arrange that the direction of the positive current is such that the capillary mercury is the cathode, otherwise mercurous sulphate might be formed in the capillary tube on account of the passage of a rather large current. *For the same reason, the short-circuiting key should only be opened for as brief periods as possible.*

Fine instruments may be purchased filled, adjusted and sealed ready for use (Figs. 12 and 106).

22. **Strength of Acids.**

(*a*) *By Taste.* Prepare a number of N/10 solutions of acids, *e.g.* hydrochloric, acetic and boric acids. Dry the side of the tongue and apply a small quantity of the acids in turn with a camel's-hair brush, rinsing the mouth out after each application. Place them in their order of sourness. Dilute each of them ten times and repeat the experiment.

(*b*) *By hydrolysing power.* Take three test tubes and put 2 c.c. of a 0·5 M solution of cane sugar in each. Add to each respectively 5 c.c. of one of the acids prepared above, *e.g.* N/10HCl, N/10HA and N/10HBO_3, and place all three in boiling water at the same time. Leave them there for $1\frac{1}{2}$ to 2 minutes. Remove all together and cool. Add 5 c.c. N/10NaOH to each tube, mix and then add 5 c.c. of *Benedict's* qualitative *sugar reagent.* Boil and compare the amount of copper reduction in each tube.

(*c*) *By indicators.* (α) Prepare a series of graded concentrations of a strong acid like hydrochloric and a similar series of a weak acid such as acetic, *e.g.*:

Take seven test tubes, and in every tube except the first put 9 c.c. of dis-

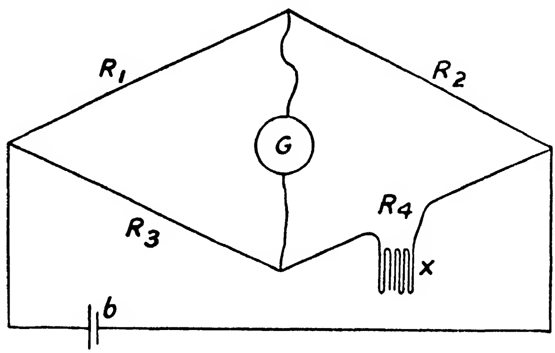


FIG. 107.—Diagram of conductivity apparatus.

tilled water. In the first tube put 10 c.c. of N acid. Transfer 1 c.c. of this to the second tube, mix and transfer 1 c.c. of the N/10 acid so prepared to the third tube, and so on, rejecting the 1 c.c. removed from the last tube. You will then have a series of tubes containing 9 c.c. of (1) N, (2) N/10, (3) N/100, (4) N/1,000, (5) N/10,000, (6) N/100,000, (7) N/1,000,000 acid.

To each tube add a few drops of B.D.H. " Universal " indicator, mix and compare the colours produced with those of the standard labelled tubes provided. Why does tube 3, which gives a reading of about *p*H 2 with hydrochloric acid, only give a *p*H of between 3 and 4 when acetic acid is used ?

(β) Determine the same range more accurately, using *thymol blue* (*p*H 1·2–2·8), *bromo-phenol blue* (2·8–4·6), and *methyl red* (4·2–6·3) in turn as indicators.

(*d*) *By conductivity.* Comparison of the electrical conductivity of equimolecular solutions of mineral and organic acids. Conductivity, being the reciprocal of resistance, obviously can be measured by a resistance method, *e.g.* by the Wheatstone bridge (Fig. 107). The current from *b* is divided between two circuits (1) by R_1 and R_2 and (2) by R_3 and R_4 (where R = resistance in ohms). The amount of current travelling by these circuits is such that the drop of potential in both is the same. If then a lead be taken from the

junction between R_1 and R_2 and connection made with the R_3, R_4 route so that $R_1/R_2 = R_3/R_4$, then no current will flow through the lead as shown by the indicator (G), a galvanometer, lamp, telephone receiver, etc. Thus R_1, R_2 and R_3 being known, R_4 can be found. In practice, the lead fixed between R_3 and R_4 terminates in a jockey-piece which slides along a platinum, iridium, or nickelin wire of uniform resistance and a metre long, constituting R_1, R_2. The length of R_1 is read from an underlying scale when G indicates that no current is passing.

Owing to polarisation occurring at the electrodes when a steady current is passed through an electrolyte, it is necessary to employ an alternating current which renders a galvanometer useless as an indicator. The alternating current is usually got from the secondary terminals of an induction coil. As an indicator, one may use a telephone receiver or a small A.C. pea lamp.

Experiment (*a*). Rough Demonstration. Fit up a Wheatstone bridge, using a 4-volt accumulator with switch as b, and a pea lamp as G. R_3 is a resistance box and R_4 or x is a simple type of conductivity cell, *e.g.* a beaker containing the solution to be tested with two sheet-silver electrodes. Find the point on the metre wire when the light from the lamp is at its minimum $= x$. Then conductance

$$c = \frac{x}{R_3(100 - x)},$$

and the molecular conductivity is equal to $C\phi$, where ϕ is the volume in cubic centimetres in which 1 mole is dissolved. Suitable solutions = 1/16 molar hydrochloric, acetic, and benzoic acids; 1/8 molar NaCl and glucose. (*N.B.*—Keep the switch from b open as much as possible to prevent polarisation of the electrodes of x.)

(β) With the same apparatus the neutral point of a titration may be determined. Place 20 c.c. of N/50NaOH in the conductivity cell and arrange the resistance box (R_3) so that the bridge reading is about 50 cm. From a burette run in a standard (N/50) solution of H_2SO_4 and mix as in titration, determining the point of balance on the wire after each addition. It will be found that the balance point will first tend towards the zero end of the scale and later will move in the reverse direction. The point at which it changes direction is the electrotitrametric neutral.

For any but very rough readings, many precautions have to be observed. These will be found in any book on practical physical chemistry. Instead of a lamp, the capillary electrometer may be used as an indicator.

23. **Principle of Measurement of H-ion Concentration by Potentiometer.** The principle is much the same as that of the conductivity measurements (Fig. 107), only instead of having a single source of potential and an unknown resistance, one has known resistances and two sources of E.M.F., one of which is of unknown value.

In Fig. 107, b may be taken as a cell of standard E.M.F. sending its current through the wire bridge, R_1—R_2. If we lead into R_1—R_2 the wires from another battery x, taking care that the direction of the difference of potential is the same, *e.g.* both negative poles leading to R_1, as the fall of potential along R_1—R_2 is regular, we can readily divide the wire so that the difference of potential between the point of entrance and exit of the current from x is equal and opposite to the difference of potential between the same points caused by the standard cell, *i.e.* the galvanometer or electrometer will indicate no E.P.D. The cell x is of interest. It consists of two half-cells or electrodes. One of these is the ordinary standard calomel electrode, *i.e.* an electrode of

mercury covered with HgCl in the presence of a definite concentration of KCl. The other half-cell is a hydrogen electrode, *i.e.* platinum black laden with hydrogen and immersed in a solution containing H-ions. The difference of E.M.F. between electrode and solution depends on the concentration of H-ions in the latter.

The difference of potential between the calomel and normal hydrogen electrode can be ascertained. This value is subtracted from the total E.M.F. of the cell to give a value from which the *p*H may be calculated.

24. **Alterations of the Surface Tensions of Oil-Water Interface by Alterations in Hydrogen Ion Concentration** (Hartridge and Peters, *Jour. Physiol.* LIV., Proc. XLI.).

Oil free from fatty acids or soaps is essential. Pure olive oil, castor oil, or cod-liver oil may be freed from these bodies by boiling for a number of hours with frequent changes of a considerable excess of tap water. A series of test tubes 6 × $\frac{5}{8}$ in. each receives 5–10 c.c. of the treated oil. Into each is put a capillary tube 4 in. long, open at both ends. The test tube is almost filled with the fluid to be tested. Try solutions having a *p*H of 9, 8, 7, 6. Measure the height to which the oil rises in the capillary.

25. **Buffer Solutions.**

(A) Sørensen's Phosphate Buffers. Two solutions are required, (*a*) M/15 solution of KH_2PO_4 (9·078 gm. per litre). The salt should be chemically pure, and its solution should be water clear and free from even traces of chloride and sulphate.

(*b*) M/15 solution of $Na_2HPO_4 . 2H_2O$ (11·876 gm. per litre). The salt is prepared by exposing to the air the recrystallised $Na_2HPO_4 . 12H_2O$. After two weeks' exposure on a porous plate, dissolve the salt in water and test for chloride and sulphate: 8 c.c. of (*a*) KH_2PO_4 + 2 c.c. of (*b*) Na_2HPO_4 $2H_2O$ gives a solution of *p*H 7·381.

The water used for making these solutions must be free from CO_2. This is obtained by boiling the glass-distilled water in a hard glass flask for at least 5 minutes, and then fitting the flask with a bored rubber stopper carrying a soda-lime tube so that the water may cool in an atmosphere free from CO_2. The solutions may be kept in a hard glass double-necked bottle, of which one neck is connected to a soda-lime tower fitted with a blow-ball, and the other neck to the side tube of a burette. The burette is provided with a soda lime tube at the upper end. It is as well to coat the inner surface of the bottle with paraffin wax (see p. 557).

(B) *Clark and Lubs.* Five solutions are necessary to provide a complete *p*H range.

(i.) 0·2 M *acid potassium phthalate.* Recrystallise the salt from distilled water and dry at 110° C. for some hours. Dissolve 40·828 gm. in distilled water and make up to a litre.

(ii.) 0·2 M *acid potassium phosphate.* Recrystallise the salt from distilled water and dry as above. Dissolve 27·231 gm. in distilled water and make up to a litre.

(iii.) 0·2 M *boric acid in* 0·2 *M KCl.* Dry the boric acid in air on a porous plate. Use pure ignited KCl. Dissolve 12·4048 gm. of HBO_3 and 14·912 gm. of KCl in distilled water and make up to a litre.

(iv.) 0·2 N *sodium hydroxide.* Standardise this solution against weighed amounts of pure potassium phthalate, using phenolphthalein as indicator.

(v.) 0·2 N *hydrochloric acid.* Prepare this from a freshly distilled 20 per cent. solution and standardise it against the standard N/5 soda, using methyl red as indicator.

*p*H Range 4·49–9·18

Indicator, B.D.H. (Universal).

More accurate determinations may be made with one of the indicators given in the next section.

*p*H.	c.c. M/15 Na_2HPO_4.	c.c. M/15 KH_2PO_4.
4·49	0	10·0
4·94	0·1	9·9
5·29	0·25	9·75
5·59	0·5	9·5
5·91	1·0	9·0
6·24	2·0	8·0
6·47	3·0	7·0
6·64	4·0	6·0
6·81	5·0	5·0
6·98	6·0	4·0
7·17	7·0	3·0
7·38	8·0	2·0
7·73	9·0	1·0
8·04	9·5	0·5
8·34	9·75	0·25
8·68	9·9	0·1
9·18	10·0	0

I. *p*H Range 2·2–3·9

Place 50 c.c. of solution (i.) in a number of flasks, add a drop of either *thymol blue* or brom-phenol blue to each and various amounts of solution (v.) as under:

c.c. N/5 HCl .	46·7	42·5	39·6	37·0	32·95	29·6	26·42	22·8	20·32
*p*H . . .	2·2	2·3	2·4	2·5	2·6	2·7	2·8	2·9	3·0

c.c. N/5 HCl .	17·7	14·7	11·8	9·9	7·5	5·97	4·3	2·63	1·0
*p*H . . .	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9

Make each up to 200 c.c. with distilled water.

II. *p*H Range 4–6·3

To 50 c.c. of solution (i.) add a drop of either brom-phenol blue, methyl red or brom-cresol purple, and the number of cubic centimetres of solution (iv.) given below. Make up to 200 c.c.

c.c. N/5 NaOH .	0·4	2·2	3·7	5·17	7·5	9·6	12·15	14·6
*p*H . . .	4·0	4·1	4·2	4·3	4·4	4·5	4·6	4·7

c.c. N/5 NaOH .	17·7	20·95	23·85	27·2	29·95	32·5	35·45	37·7
*p*H . . .	4·8	4·9	5·0	5·1	5·2	5·3	5·4	5·5

c.c. N/5 NaOH .	39·85	41·9	43·0	44·55	45·45	46·2	47·0	48·1
*p*H . . .	5·6	5·7	5·8	5·9	6·0	6·1	6·2	6·3

III. *p*H . 5·8–8·0 (Physiological Range).

Solutions: 50 c.c. solution (ii.), and *x* c.c. of N/5NaOH.
Indicators. Brom-cresol purple; brom-thymol blue; or Phenol red.

Dilute to 200 c.c.

x c.c.	*p*H	*x* c.c.	*p*H	*x* c.c.	*p*H
3·72	**5·8**	**17·8**	**6·6**	**39·5**	**7·4**
4·7	**5·9**	**21·0**	**6·7**	**41·2**	**7·5**
5·7	**6·0**	**23·65**	**6·8**	**42·8**	**7·6**
7·4	**6·1**	**26·50**	**6·9**	**44·2**	**7·7**
8·6	**6·2**	**29·63**	**7·0**	**45·2**	**7·8**
10·19	**6·3**	**32·5**	**7·1**	**46·0**	**7·9**
12·6	**6·4**	**35·0**	**7·2**	**46·8**	**8·0**
16·0	**6·5**	**37·4**	**7·3**	—	—

IV. Range 7·8–10·0

Place 50 c.c. of solution (iii.) in a number of 200 c.c. flasks. Add to each a drop of one of the indicators cresol red or thymol blue and the number of cubic centimetres of solution (iv.) given below.

c.c. N/5 NaOH .	2·61	3·3	3·97	4·8	5·9	7·3	8·5	10·4
*p*H . . .	7·8	7·9	8·0	8·1	8·2	8·3	8·4	8·5

c.c. N/5 NaOH .	12·0	14·3	16·3	19·0	21·3	24·3	26·7	29·95
*p*H . . .	8·6	8·7	8·8	8·9	9·0	9·1	9·2	9·3

c.c. N/5 NaOH .	32·0	34·5	36·85	39·0	40·8	42·5	43·9	
*p*H . . .	9·4	9·5	9·6	9·7	9·8	9·9	10·0	

To demonstrate the acid-combining power of a "buffered" solution. Take 50 c.c. of the above mixture (*p*H = 7·8) or prepare a solution containing about 0·25 per cent. sodium bicarbonate. Place this in a tall cylinder and in another similar vessel place the same quantity of water.

Add about 20 drops of neutral red (0·05 per cent. in alcohol) to each. Add N/10 NaOH to the water till it has approximately the same pH as the bicarbonate. Titrate both with N/50HCl to the same end point.

26. **Dialysis** (see pp. 557–560).

(1) In using any dialyser it is advisable to look to the following points :—

1. Test for leaks.
2. See that neither the preservative nor the fluid to be dialysed act on the substance of the membrane, *e.g.* bile pigment increases the permeability of collodion. (Bile may be dialysed through a double dialyser, *i.e.* a collodion tube suspended in a larger tube of the same material. Blood pigment occasionally presents the same difficulty.)
3. If the dialysate is wanted as well as the dialysed fluid, dialysis must be carried out by changing the external fluid from time to time.
4. If it is not necessary to keep the external fluid for examination, rapid dialysis may be obtained by keeping up a continuous flow of water in the outer vessel. This is most conveniently done by placing the dialyser or a series of dialysers in a sink, the level of water in which may be adjusted by means of a wide glass tube running through the waste plug. The water supply is led to the bottom of the sink.

(2) (*a*) Dialyse (*a*) Egg albumin + Sodium Chloride. (*b*) Starch + Iodine + HCl. (*c*) Starch + Glucose. Test dialysate for both constituents.

(*b*) Using a glass dialyser with a collodion membrane dialyse a mixture of either congo red, litmus or alizarin and hydrochloric acid.

(*c*) Dialyse some blood serum. What is the precipitate ? How do you explain this ?

(*d*) **An acid Perfusate from an Alkaline Solution**. Prepare some colloidal ferric hydrate either by dialysing 5 per cent. ferric chloride or by gradually adding 1 c.c. of 30 per cent. ferric chloride to 25 c.c. of boiling water. Put 10 to 15 c.c. of this sol into a dialysing thimble suspended in an Erlenmeyer flask, containing distilled water and some indicator (litmus or methyl red). Estimate the concentration of iron in the wash-water by abstracting, at each change of water, 10 c.c. of the fluid and adding K_4FeCy_6 solution. When the iron content becomes very small (after 48 hours) add some HCl to the colloid. Note the increase in the diffusibility of the Fe. How can you explain this ? Why does an acid perfusate come from an electro-negative sol ?

27. **Faraday-Tyndall Phenomenon** (p. 79).

Arrangement of apparatus. The fluid to be examined is placed in a prismatic cell (small flat-sided specimen jar, say $5 \times 5 \times 2\frac{1}{2}$ in.). One large face of the cell is covered with a black velvet curtain. Light from any optical projector is passed through the cell in a plane parallel to the long side. A lens is interposed so that the focus falls about the middle of the cell forming a cone. A darkened room is essential for any but individual demonstrations.

A. (1) Fill the cell with water and show that the beam is hardly shown. (With conductivity water the beam is not seen, see preparation of special water, p. 557.)

(2) Dust lycopodium or puff smoke into the beam outside the cell to show how small particles in air affect the visibility of the light.

(3) Add about 1 c.c. of an egg albumin solution to the water in the cell. Why is the cone bluish in tinge ?

(4) Replace the solution with another containing a red gold sol. Why is the cone green ?

(5) Try also 1 per cent. starch, 1 per cent. mastic, a saturated solution of cane sugar or milk whey.

B. Polarisation of the Tyndall Cone. Fill the cell with a mastic sol. Cut down the light from the lantern to the minimum, which will still give a well-defined cone. Examine the cone with a Nicol prism (*q.v.*) held at right angles to the beam of light and rotate the prism. The cone of light will become very dim *twice* in every complete revolution of the prism. That is, the light is partially plane polarised.

Another similar cell should be ready to be substituted for the one containing the mastic sol, or should be placed in series with it and nearer to the source of light. Fill the second cell with a fluorescent solution such as that of quinine bisulphate or of fluorescein or of eosin. Fluorescent solutions produce a cone, but no polarisation of the light.

28. **Ultra-microscope.**

The "slit" ultramicroscope and the cardioid condenser require special lighting, but the coarser ultramicroscopic particles can be seen by the use of a dark-ground condenser such as that shown in Fig. 15 on p. 80 (or Watson's or Zeiss' "Paraboloid," Reichert's "Table," Jentzsch's "Concentric," or Watson's "Nelson Cassegrain"). The last-named condenser can be used with any oil immersion objective utilising its full aperture without a funnel-stop in the objective. It is effective through the thickness of an ordinary microscope slide.

Paraboloid Condensers (Watson or Zeiss). These condensers are simply substituted for the optical part of the Abbe condenser. A funnel-stop must be placed in the oil immersion objective to reduce their aperture below 1·0. The following points require attention.

(i.) The paraboloid must, *in every case,* have oil between it and the microscope slide. All air bubbles must be excluded, and sufficient oil used to maintain a perfect contact between slide and condenser.

(ii.) The condenser must be *accurately* centred.

(iii.) The fluid under examination should be placed on the slide in as thin a layer as possible.

(iv.) The illumination should be as brilliant as possible. Good results will be obtained by the use of an Ediswan "Point-o-lite" with bull's-eye condensing lens.

(v.) The slides and coverslips must be clean. The best method is to immerse them in hot bichromate-sulphuric acid mixture for 5–10 minutes. Rinse thoroughly in distilled water. Pick up the slides and slips one at a time with forceps; shake off the bulk of the water and place in dry alcohol till required. When required withdraw a slide from the alcohol (*using forceps*) and burn off the adherent alcohol in a spirit lamp. *As soon as the slide has cooled* place it on the oil on the condenser. The slips may be treated in the same way, or, if they crack readily, the alcohol may be removed by evaporation near the flame. The cover slip, *as soon as it has cooled,* is placed gently on the drop of sol to be examined. *At no time should the slips or slides be touched by hand or by cloth.*

A. Mastic sols, gold sols and suspensions like gamboge and India ink show up well.

B. **Brownian Movement** (p. 82 and Fig. 16). (1) Clean slides and cover glasses in hot potassium bichromate-sulphuric acid mixture, rinse in distilled water and then in two changes of alcohol. Keep till required in alcohol (see Experiment 28 above).

(2) When ready to examine a sol, withdraw a slide from alcohol (using forceps) and evaporate dry over a clean flame. Cool. A large drop of sol free from air bubbles is placed on the centre of the slide. The cover

glass, prepared like the slide, is gently placed on the drop. Use a good electric light, such as the Ediswan " Point-o-lite," and focus on the central portion of the fluid. A 1 per cent. suspension of gamboge shows the Brownian movement well.

29. **Diffusion.**

(a) *Colloid into colloid.* Two-thirds fill four test tubes with sterile 3 per cent. gelatin. Plug tubes with cotton-wool. When the gel has set firmly, place 0·1 per cent. sols of (1) Congo red, (2) Prussian blue, (3) colloidal iron, and (4) black India ink, one to each tube. Keep tubes plugged with cotton-wool and in a cool place. Examine after two days and again after two weeks.

(b) *Crystalloid into colloid.* Prepare a number of tubes of gelatin as above. When the gelatin has set, pour various coloured solutions over the jelly and examine as above. Try copper sulphate, methylene blue, methyl violet, picric acid.

(c) *Diffusion of acid into gelatin.* Mix a 4·5 per cent. gelatin sol with just alkaline phenolphthalein. When set cover with acidified night blue. The acid diffuses rapidly from the dye. Two coloured layers separated by a colourless layer are produced in a very short time.

(d) *Electrical diffusion* (p. 86). Fit up a U-tube with an electrode of platinum- or silver-foil rolled cylindrically at the top of each tube. Fill the tube two-thirds full with 3 per cent. gelatin containing a trace of citric acid, and allow to stand overnight to form a gel. Fill one limb with a coloured electrolyte (*e.g.* $CuSO_4$), the other with acidulated water. Determine roughly the rate of diffusion (2 hours). Then pass a current through the tube (lighting supply with a lamp in circuit) and note rate of diffusion (2 hours). Reverse the direction of the current for 2 hours or more and note changes. Try various electrolytes and find which are forced into the gel at the cathode and which at the anode.

30. **Adsorptive Stratification.** (Liesegang Phenomenon, p. 86.)

(a) Four grams of gelatin are dispersed in 100 c.c. of water and 2 c.c. sat. potassium bichromate are added to the sol. The mixture is poured on clean glass plates to form a thin layer, about 0·45 c.c. per sq. in. of surface being allowed. The plate is supported on a horizontal surface and the sol allowed to set; 10–15 minutes will be required. A large drop of 20–30 per cent. silver nitrate is placed in the centre of the plate, preferably by allowing five successive drops of about 0·1 c.c. each to fall on the same spot from a small pipette. The drop should have a clean circular outline. The plate is kept in the dark for 24–48 hours. At the end of this period any traces of the original drop may be removed with a pointed strip of filter paper, and the gel is then allowed to dry. (1) Use commercial gelatin. (2) Do not disturb the plate after adding $AgNO_3$, till excess has been removed. (3) A trace of citric acid (5–10 drops of 5 per cent. solution to 100 c.c. of sol.) gives wider rings.

(b) (i.) Fill a test tube to about two-thirds with the bichromate-gelatin mixture mentioned above. When the gelatin has set fill the remaining one-third with (approximately) 10 per cent. silver nitrate. Keep in the dark and reasonably cool.

(ii.) Prepare a gelatin sol containing 3 gm. of gelatin and 80 c.c. of water. When solution is complete add 20 c.c. of a 50 per cent. solution of $MgCl_2 . 6H_2O$ and mix. Allow to set and then cover with concentrated ammonia. Cork well. Examine after a week. The rings are scarce and well separated.

(c) Saturate the gelatin in Experiment (b) (ii.) above with a capillary active substance like quinine. Why are no rings formed ?

(*d*) *Dead-space experiment* (p. 87). Fill a glass tube, of about $\frac{1}{2}$ in. diameter and 6 in. in length, and open at both ends, with a 5 per cent. gelatin sol to which sufficient silver nitrate has been added to give a Normal solution. Immerse the tube, when the gelatin has set, in a 10–15 per cent. solution of sodium chloride. Ring formation, starting from each end, stops before the middle is reached.

(*e*) *Rings formed between gases in air.* Put a cork into a 1 oz. bottle. Through the cork pass a glass tube about a metre long and between 1 and 2 cm. in diameter. Plug the upper end of the tube loosely with glass wool. Put a few cubic centimetres of HCl into the bottle and moisten the wool with concentrated ammonia. Leave overnight.

31. Determination of the Relative Viscosity of a Liquid.

When a liquid flows through a narrow tube the velocity of flow depends mainly (α) on the force producing the flow and (β) on the resistance to flow produced by the viscosity or internal friction of the liquid. In Chap. XXV. we considered the shearing of the different layers of the blood stream. The liquid, we saw, could be regarded as made up of a number of concentric tubes sliding past one another. When the liquid is moving through the narrow tube there will be, under constant conditions, a constant difference in velocity between the different tubular layers. The force per unit area necessary to maintain this condition is proportional to the difference of velocity, v, of two adjacent layers, and inversely proportional to their distance apart, x.

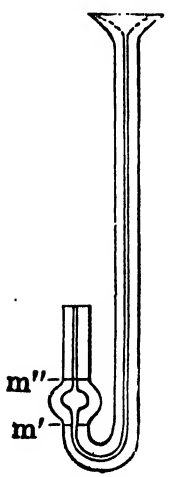


FIG. 108.—Viscosimeter (*Hawksley*).

Briefly, Force $= \eta \times \frac{v}{x}$, where η is the *coefficient of viscosity*, which is the force per unit area when $v = x$. If, now, the *quantity* of fluid and the *pressure* be kept constant and the *time* observed which the fluid takes to travel a certain *distance*, the viscosity of two liquids with densities s' and s'' with times of flow t' and t'' will be as

$$\eta'/\eta'' = s't'/s''t''.$$

Neglect of the difference of density introduces an error of less than 1 per cent. and materially simplifies the operation, *i.e.*

$$\eta'/\eta'' = t'/t''.$$

Apparatus (Denning-Watson Viscosimeter). The instrument is a modification of the Ostwald-Poiseuille viscosimeter. It consists of a U-tube with a long and a short arm (Fig. 108). The long arm (6 cm. in length) is blown out at its free end into a cup-shaped receiver with a thin edge.

On the short arm (2 cm. in length) there is a small elliptical bulb, the capacity of which is defined by the two lines m' and m''.

Method of use. The receiving cup at the end of the long arm is filled (and kept filled) with the fluid to be tested, which passes down the capillary tube. A stop-watch is started when the fluid reaches the point m' and stopped when it reaches m''. The time taken is compared with the time reading of water which is recorded on the back of the tube used. Denning and Watson urge attention to the following points. (1) The tubes should be scrupulously clean and *perfectly dry*. (2) The viscosimeter and the fluid must be at the same fixed temperature. (3) The receiver of the instrument must be kept *filled* with the fluid, for pressure-height must be kept constant, *i.e.* compared with

water. (4) The movement of very viscous fluids may be initiated by slight suction applied to the short arm. (5) Clean the tubes immediately after use. They are best cleaned by a strong reagent like conc. HNO_3 (depending on the fluid last measured), followed in quick succession by distilled water, alcohol and ether. Hot air is passed through the tube to dry it.

(*a*) Compare the viscosities of (i.) water, (ii.) 1 per cent. sodium chloride (iii.) colloidal iron, (iv.) 1 per cent. starch, and (v.) 1 per cent. gelatin.

(*b*) *Effect of mechanical agitation on the viscosity of a gelatin solution.* Prepare a 0·5 per cent. solution of gelatin and divide it into two parts *A* and *B*. Leave *A* undisturbed for 12–24 hours. Cause *B* to be agitated for a few minutes before the final experiment by sucking it up and blowing it down a capillary pipette or by blowing bubbles through it. Compare the viscosities of *A* and *B*.

(*c*) *Effect of concentration on viscosity.* Prepare various concentrations of gelatin and of aqueous acacia resin, *e.g.* 0·5 per cent., 2·5 per cent., 5 per cent., 10 per cent., and 15 per cent. Compare the viscosities. (The gelatin experiments will have to be carried out at about 20–30° C.)

(*d*) *Effect of temperature on the viscosity of gelatin.* A 5 per cent. solution is divided into three tubes, and after standing overnight the tubes are put for *half an hour* (*a*) into a freezing mixture, (*b*) into water at 40° C., and (*c*) into water at room temperature. Compare viscosities.

(*e*) *Effect of electrolytes on the viscosity of gelatin.* A 1 per cent. solution of gelatin is taken, and the following dilutions and admixtures prepared :

(i.) 10 c.c. 1 per cent. gelatin + 10 c.c. water.
(ii.) ,, 9 c.c. water + 1 c.c. $\frac{N}{10}$ HCl = $\frac{N}{100}$.
(iii.) ,, 10 c.c. $\frac{N}{10}$ HCl.
(iv.) ,, 10 c.c. $\frac{N}{10}$ NaOH.
(v.) ,, 10 c.c. $\frac{N}{100}$ NaOH.
(vi.) ,, 10 c.c. N Na_2SO_4.
(vii.) ,, 10 c.c. N NaCNS.

(*f*) *Effect of varying the* pH *on the viscosity of acacia.* Make a 5 per cent. aqueous solution of acacia resin and dilute it with hydrochloric acid as under to give the following concentrations of H^+ in a 2·5 per cent. solution of acacia. Take six test tubes and put 5 c.c. of water in each. Leave the first tube as control, and add 5 c.c. 2 N . HCl to the second tube, mix and transfer 5 c.c. of the mixture to the third tube, and so on, rejecting the 5 c.c. of the mixture taken from tube 6. Now add 5 c.c. of the 5 per cent. acacia solution to each tube.

Tube No.	1	2	3	4	5	6
Water, c.c. . .	5	5	5	5	5	5
Acid, c.c. . .	0	5	5	5	5	5
Acacia, c.c. .	5	5	5	5	5	5
Normality . .	0	N	N/2	N/4	N/8	N/16

Acacia per cent., 2·5 in all tubes.

Determine the viscosity of the mixture in each tube. The lowest viscosity will be found in tubes 4 and 5. If a new series of tubes is prepared with concentrations of acid lying between these values, *e.g.* 0·25 N to 0·125 N, the lowest reading will be obtained with about 0·2 N . HCl.

32. Determination of the Isoelectric Point of a Protein (p. 91).

(*a*) *Preparation of solutions of definite* (*temporary*) *p*H. Seven clean, dry tubes are treated as under.

Tube No.	1	2	3	4	5	6	7	
Water, c.c. .	2	9	9	9	9	9	9	
Acetic acid, c.c. .	16	9	9	9	9	9	9	+9
*p*H . . .	3·8	4·1	4·4	4·7	5·0	5·3	5·6	

Place 2 c.c. of distilled water in tube 1 and 9 c.c. in each of the other tubes. To tube 1, add 16 c.c. N/10 acetic acid. Mix and transfer 9 c.c. of the mixture to tube 2. Mix and transfer 9 c.c. to the next tube, and so on, rejecting the 9 c.c. withdrawn from tube 7.

Determination of the isoelectric point of casein. Put 1 c.c. of a casein sol (*q.v.*) into each of seven clean, dry test tubes. Add the contents of acid tube 1 (*p*H 3·8) to the first casein tube, tube 2 (*p*H 4·1) to the second casein tube, etc. Shake each of the tubes and record your observations on a chart as below. — = no change, O = opalescence, + = precipitate.

Tube No.	1	2	3	4	5	6	7
On mixing	0	0	0 0	0 0 0	0 0	0	—
After 10 minutes . .	0	0 0	+ +	+ + +	0 0 0	0	—
After 20 minutes . .	0	0 0	+ +	+ + +	+	0	—
*p*H	3·8	4·1	4·4	4·7	5·0	5·3	5·6

Tube 4 (*p*H = 4·7) shows the greatest change, and so it is inferred that the isoelectric point of casein lies near that value.

(*b*) *Determination of the isoelectric point of gelatin.* Make up a series of buffer solutions of *p*H 4, 4·2, 4·4, 4·6, 4·8, 5, 5·5 from the phthalate series given in Experiment 25. To each of a series of seven clean boiling tubes containing 10 c.c. of these buffers, add 1 gm. of powdered gelatin and 1 c.c. of M/128 potassium ferrocyanide solution. To another similar series, add 1 c.c. of M/128 copper acetate solution. After the gelatin has been in contact with the ferrocyanide or the copper solution at a definite *p*H for about an hour, pour off the supernatant fluid, wash several times with *cold* water to remove any metal salt not combined with the gelatin and dissolve the gelatin by adding warm water to each tube and immersing the tubes in warm water (40° C.). The volume is made up to 50 c.c. Allow to stand for two or three days. The tubes of the first series, in which the *p*H was less than 4·7, turn blue because the gelatin forms the cation of a salt in which $Fe(CN)_6$ is the

anion. The latter forms a blue *ferric* salt on standing for a few days. The other tubes in this series are colourless. Similarly, the second series shows a blue range from pH 4·8–5·5 due to the combination of the gelatin with the copper to give a copper gelatinate. From these experiments one infers that at a pH between 4·6 and 4·8 gelatin acts neither as a cation nor as an anion. 4·7 is, therefore, approximately the isoelectric point of gelatin.

(*c*) *Determination of the isoelectric point of gelatin by the alcohol precipitation method* (Pauli). Prepare a series of buffer solutions of the sodium acetate-acetic acid series, having a range about the isoelectric point of gelatin, *e.g.* 4·1 to 5·3 as follows:

Take five boiling tubes and put 8 c.c. of distilled water in the first and 5 c.c. of distilled water in each of the others. To tube 1 add 2 c.c. of N acetic acid and mix. Transfer 5 c.c. of this N/5 acid to tube 2 and mix, and so on, rejecting the 5 c.c. of N/80 acid removed from tube 5. That is, a series of tubes having 5 c.c. of fluid of the following pH values has been prepared:

Tube No.	1	2	3	4	5
pH . . .	4·1	4·4	4·7	5	5·3

To each tube add 2 c.c. of N/10 sodium acetate. Mix and add 2 c.c. of a 1 per cent. gelatin sol. Shake. Now slowly add methylated spirit to tube 3 till a cloudiness is *just* visible. This will require about 8 c.c. Add this amount to each of the other tubes and leave for half an hour. In which tubes is turbidity most pronounced?

33. Osmotic Pressure of Gelatin.

(1) Fit up an osmometer with a collodion membrane (as described on p. 557). Fill the collodion sac with a 1 per cent. gelatin sol and place the osmometer in water up to the level of the rubber stopper. It is necessary to keep the apparatus and its contents at about 16–20° C. to prevent gelatinisation. In about 20–30 hours the level of the fluid in the vertical tube will be steady and there ought to be about 40 mm. of solution above the level of the fluid outside.

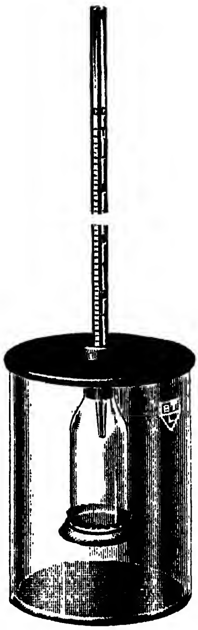


FIG. 109.—Osmometer with collodion or parchment membrane (see text).

(2) A simple comparative experiment to illustrate the low osmotic pressure of gelatin at its isoelectric point may be carried out by means of three thistle funnels (Fig. 5*b*). Over the mouth of each funnel is placed a collodion membrane *B* (in the same way as is described in the preparation of a collodion dialyser). Into the first osmometer put enough acid gelatin to come above the wide part into the tube. Charge the second one similarly with isoelectric gelatin and the third with alkaline gelatin, and place all three vertically in the same water. After a day one can see that tube 2 has practically no osmotic pressure, while the other two exhibit a pressure of about 70 mm. of solution. The results are not quite accurate because the unprotected collodion forming the floor of this simple osmometer is distensible and yields more with the greater hydrostatic pressure. Another disturbing factor is the electrostatic attraction of the gelatin for the Cl ions and the Na ions, whereby both Cl and Na are prevented from diffusing freely through the membrane (see Donnan Equilibrium, p. 143). They thus also exert an osmotic effect and exaggerate the rise in the first and third tubes.

34. Cataphoresis.

(*a*) *Macroscopic observation of the movement of colloids in an electric field.* Material, etc., required :

U-tube, 15–25 cm. high × 2–3 cm. inside diameter, fitted with rubber corks pierced by two holes (p. 91, Fig. 19). In one hole in each cork put a short length of glass tubing to act as a vent for gas and water. The other hole carries the electrodes, preferably of platinum or silver, but copper will answer quite well. These electrodes may be flat, but more rapid results will be given if the metal foil is rolled into cylindrical form. (Diameter of cylinder 2 mm. less than that of the tube.)

Egg white sol (p. 562). Fill the lower third of the U-tube with the neutral solution, and gently drop a small disc of smooth writing paper on top of each surface. With a pipette fill each limb with distilled water, so that there is a clear demarcation at the water sol interface. Gently insert the corks carrying the electrodes. Allow to stand undisturbed for 15 minutes. Now pass at least 110 volts D.C. across the electrodes for half an hour or so and note the alteration in the levels of the sol in each limb. With a D.C. voltage of about 200 the gradient in this tube would be about 5V/cm., so that in 15 minutes the level of the sol should move quite distinctly. Reverse the direction of the current for a similar time. Repeat the experiment with some egg white sol which has been made (*a*) slightly acid with acetic acid, or (*b*) faintly alkaline with dilute sodium hydrate.

A rough demonstration of the movement of colloids in an electric field may be given by fitting up a small cylindrical zinc water bath (or even a cocoa tin) with a central cylindrical roll of copper foil suspended from a glass or wooden cross-piece. Fill the jar with the colloid to be studied and leave overnight. Positively charged colloids will adhere to the copper foil, while those carrying a negative charge will be found in association with the zinc.

Colloids to try. 1. Egg white (*a*) neutral to litmus, slightly electronegative, (*b*) acidified with acetic acid, (*c*) made just alkaline with sodium hydrate. 2. 1 per cent. gelatin, (*a*) isoelectric, (*b*) electropositive, (*c*) electronegative. 3. 0·2 per cent. night blue. 4. 0·2 per cent. alkali blue. 5. Mixture of 100 parts of 2 per cent. alizarin red and 2 c.c. saturated picric acid.

(*b*) *Microscopic observation of movement of colloids in an electric field* (Fig. 20, p. 91, and letterpress, p. 92). The electrodes (Fig. 20) are two strips of platinum- or silver-foil fastened parallel to one another about 16 mm. apart (Chatterton's compound is an excellent fixative). The slide is placed on a microscope with a paraboloid condenser (or with a small stop) and the lighting, etc., arranged to suit the particular type of condenser used.

A large drop of the sol under examination is placed in the centre of the space between the electrodes, making contact with them and covered with a $\frac{7}{8}$ in. slip. After 10 minutes the microscope is focussed on the central layer of liquid (particles not in contact with glass and free to move) and a current of 4–5 volts (keep amperage low) passed between the electrodes. Determine the *sign* of the electric charge on dialysed iron sol, gold sol, night blue, alkali blue, etc. Fit an eyepiece micrometer and with a stop-watch determine the velocity of the particles in centimetres per volt per second.

35. Electric Endosomose.

(*a*) The passage of water through a membrane by electrical means may be observed in the preparation of a semipermeable copper ferrocyanide membrane when the solutions are forced into the pores of the earthenware pot by an electrical current (Experiment 5).

(*b*) A clean porous pot, fitted with a manometer and a non-polarisable

electrode, is filled with and placed in a solution of K_2SO_4 (0·05 per cent.). A current of 2–4 volts is passed so that the electrode inside the pot is the cathode. Note the rise in level of the fluid inside the pot. Note also the increase in the alkalinity of the fluid outside the pot.

(*c*) Make a collodion tube to fit one limb of the U-tube (Fig. 19).

(1) Fill both limbs with dilute K_2SO_4 solution. Mark the level of the fluid in both limbs and, using non-polarisable electrodes, pass a current of 4 volts for some time through the solution. Note that water passes towards the cathode and that the cathodal fluid becomes acid.

(2) Repeat, using tartaric acid in the collodion sac and pure water outside. Test for tartaric acid.

(3) Fill the sac with gelatin sol and leave overnight. Wash out the sol and repeat the experiments (*c*) 1 and 2.

36. **Coagulation of Sols at the Isoelectric Point** (see also Isoelectric Point).

(*a*) *By heat.* Prepare some egg albumin sol. Divide it equally into three lots. To lot 1 add 1 c.c of N . HCl for every 20 c.c. of sol. Leave lot 2 alone. Add 1 c.c. of N . NaOH to lot 3 for every 20 c.c. of sol. Place in water bath and gently warm to 60° C. Cool and examine. Filter if necessary.

Heat 5 c.c. of each lot to the boiling-point. Only No. 2 coagulates. Carefully neutralise No. 1 with N/10 NaOH and No. 3 with N/10 HCl. A precipitate is formed. Add more alkali or acid as the case may be. The precipitate dissolves. This precipitation by neutralisation is reversible. Repeat the neutralisation experiment on the remainder of 1 and 3. Filter off the precipitates and suspend each in about half a tube of water. Now heat to boiling. A coagulation forms. Coagulation is irreversible.

(*b*) *Effect of electrolytes on colloids. Method.* Take three test tubes (*clean*) for each experiment. To tube 1 add 10 c.c. of the salt solution. To tube 2 add 10 c.c. of water + 1 c.c. of salt solution, mix and transfer 1 c.c. to tube 3. To tube 3 add 10 c.c. of water + 1 c.c. from tube 2. Mix, withdraw 1 c.c. and reject it. To each tube add 1 c.c. of colloid. Mix and leave alone for one hour.

Colloids to use. (1) Suspensoid. Dialysed iron (B.D.H.) (1 in 10). (2) Emulsoid. Gum mastic (*q.v.*).

Salts to use. (i.) To show the effect of varying the anion : 2 N solutions of the nitrate, chloride, acetate and sulphate of potassium.

(ii.) To show the effect of varying the cation : 2 N solutions of the chlorides of sodium, calcium, magnesium and ammonium.

(*c*) *Mutual precipitation of colloids.* (i.) Add gum mastic to colloidal iron till precipitation occurs. Filter and test filtrate for iron.

(ii.) Add colloidal iron to diluted blood serum. Filter through cotton-wool and test filtrate for iron and for protein.

(iii.) Add tannic acid drop by drop to diluted blood serum till precipitation is complete. Now add more tannic acid. The precipitated colloid again resumes the sol form.

(iv.) *Capillary analysis* (*q.v.*).

37. **Protection of (Hydrophobic) Suspensoids by (Hydrophilic) Emulsoids.**

(*a*) Five cubic centimetres of colloidal iron (B.D.H. diluted 1 in 5) is placed in each of two test tubes. To one add 5 c.c. of 0·1 per cent. gelatin (slightly acidified) and to the other 5 c.c. of water (also slightly acidified). To each add enough of any of the salts (given in Experiment 36) to precipitate the iron. Precipitation occurs rapidly only in the tube free from gelatin. Determine how much more of the salt solution has to be added to precipitate the gelatinised iron.

(*b*) *Protective action of emulsoids* (p. 93). Two equal portions (9 c.c.) of neutral gold sol are treated (1) with 1 c.c. of a 0·1 per cent. gelatin sol and (2) with 1 c.c. of distilled water. To both are added 1 c.c. of N/1NaCl solution. Examine by pure transmitted light, *i.e.* by looking through the tubes at a uniformly illuminated screen of white paper.

(*c*) Six tubes are prepared as follows and examined as in (*b*) above :

Tube No.	1	2	3	4	5	6
Colloidal gold, c.c.	7	7	7	7	7	7
0·01 per cent. gelatin, c.c. . . .	5	4	3	2	1	0
Redistilled water, c.c.	0	1	2	3	4	5
2 N . NaCl, c.c.	4	4	4	4	4	4

38. **Adsorption.**

(*a*) *Adsorption of colloid to a surface.* Pour into a series of Erlenmeyer flasks faintly coloured suspensions of various colloidal dyes. Add a gram of blood- or bone-charcoal to each flask. Shake several times. Filter through ordinary pleated papers. Note the practically colourless filtrate obtained. Return the charcoal to the cleaned flasks and shake with water. Is any colour given off ? Now add some substance which lowers the surface tension of water, *e.g.* methylated spirit, tributyrin, etc. The colour appears in the fluid.

Dyes to try. Congo red, 0·05 per cent. ; Night blue, 0·01 per cent. ; Prussian blue, 0·01 per cent. ; Berlin blue, 0·01 per cent.

(*b*) *Adsorption of colloid to colloid. Capillary analysis.* Cut a number of strips 2 × 15 cm. from a good filter paper. (Do not take the slip from too near the edge of the sheet.) Hang two or three of these strips so that each one dips its edge into a narrow-necked vessel (Erlenmeyer flask) containing a fluid to be tested, taking care that the papers are immersed to the same and to a sufficient depth (about 2 cm.), and that glass and paper do not come in contact. Filter paper becomes negatively charged in contact with water, and, therefore, positively charged colloids will become " fixed " electrostatically at the liquid-paper interface, while negatively charged colloids will ascend with their dispersion media. (i.) Flask i. Water. ii. Aqueous night blue or Prussian blue. iii. Aqueous alkali blue. In 10–15 minutes examine the height of water and each dye on the strips.

(ii.) Flask iv. Mixture of 20 c.c. 2 per cent. aqueous alizarin red and 0·5 c.c. sat. aqueous picric acid. Leave paper hanging in this mixture for 20–30 minutes. Remove and examine. Hold over strong ammonia for a moment to make alkaline (*i.e.* to redden the alizarin). How do you account for the extremely dark band at the junction of the stains of picric acid and picric-alizarin mixture.

(*c*) *Adsorption of Salts to Colloids.* Cut a series of discs 3–4 mm. thick from a fairly concentrated gelatin gel and place them in a Petri dish containing a 2 per cent. aqueous solution of *commercial* aluminium sulphate (contains iron) and leave for some days. In three days or so the gelatin becomes tinged reddish brown (ferric salts). Now test the original solution, the solution after standing with gelatin, and the gelatin itself for iron by adding a few drops of ammonium thiocyanate to each. Note the depth of colour.

(*d*) *Electrochemical adsorption.* Prepare three solutions of a dye, one in each of three test tubes as follows:

1. 10 c.c. of dye + 1 c.c. N . H_2SO_4.
2. 10 c.c. of dye + 1 c.c. water.
3. 10 c.c. of dye + 1 c.c. N . NaOH.

Put a strip of filter paper into each tube. In a few minutes, remove the papers and wash them in *cold* water. Only one paper is permanently stained —which paper depends on the dye used.

Dyes to try (all 0·01 per cent.). Methylene blue, crystal violet, brilliant green, night blue, Nile blue, and (0·05 per cent. solution) Congo red, and 0·1 per cent. ponceau GR.

Note that this type of adsorption is irreversible.

39. **Imbibition.**

(*a*) Allow a sheet of ordinary glue to lie overnight on a moist surface so that the under portion of the glue alone is in contact with water. Note the increase in volume of the immersed portion and also the alterations in colour, opacity, elasticity, etc.

(*b*) A strip of thin sheet rubber (dental or patching rubber) about 12 in. × $1\frac{1}{2}$ in. is cut almost its whole length into two fingers of equal width. One of the divisions is immersed in a boiling tube filled with benzol, while the other half is left hanging outside. In a few minutes the immersed division imbibes benzol and swells so that it is at least half again as long and as broad as the unimmersed division.

(*c*) *Effect of the dielectric value of the imbibed fluid on the amount of swelling.* Cut a number of exactly similar strips of dental rubber, say, 6 in. × $\frac{1}{2}$ in., and suspend one in each of the following fluids in test tubes: Water, ethyl alcohol, acetone, amyl alcohol, benzol, toluol, xylol. Examine and measure the strips after about half an hour. It will be found that the rubber has not swollen in the water, slightly in the ethyl alcohol, and so on, till the largest increase is found in xylol. That is, the power of imbibition varies inversely as the value of the dielectric constant (*q.v.*).

(*d*) (i.) *To show that imbibed fluid is held under compression.* Tie a short piece of surgical *laminaria tanga* to the stem of a hydrometer (near the foot). Float in water and note the level. After some hours, again read the hydrometer scale. If the water imbibed is not under compression both readings should be the same (see experiment on p. 97).

(ii.) *Pressure by Œdometer* (Fig. 22, p. 98). Place two large teaspoonfuls of Cox's powdered gelatin in the foot of the cylindrical glass container. Replace the plunger and attach the indicator clip. The pointer should be adjusted to read zero. Add sufficient water to reach two-thirds up the cylinder. When the apparatus is examined next day it will be obvious from the drop of the pointer on the scale that the gelatin has swelled. The scale may be calibrated to give readings in cubic centimetres. Repeat the experiment, adding weights to the balance pan. For example, the addition of a weight of 1 kilo slows the rate of imbibition, 2 kilos slows it still further, and so on, till with a certain weight, say, 30 kilos, the swelling is inappreciable.

SWELLING OF GELATIN IN WATER.

Pressure (mm. Hg.)	40	80	156	240	303	377
Grams H_2O per gram of gelatin . .	2·5	2·0	1·5	1·3	1·1	0·9

Another experiment illustrating the effect of electrolytes may be tried. During the first 6–10 hours cover the gelatin in the œdometer with a M/8 sodium chloride solution. Compare the rate and amount of swelling with that in pure water. Now drain off as much of the salt solution as possible and fill up again with pure water. Both rate and amount of swelling are now increased.

(*e*) *Heat of imbibition.* Dry some commercial starch powder at 105° C. and leave to cool in a desiccator. Put about 50 gm. of this into a small beaker, insert a thermometer and read off the temperature. Add about 50 c.c. of water and stir with the thermometer. An increase of temperature of from 10–14° C. will be obtained in a few minutes.

Somewhat better results may be obtained by substituting a vacuum flask for the beaker.

Potato meal, pease meal, etc., give + 8° to + 10° C.

(*f*) *Effect of various electrolytes on imbibition.* First of all, prepare a number of standardised gelatin discs as follows. Make a concentrated solution of gelatin, adding a trace of a colloidal dye (*e.g.* Congo red) to render the gelatin easily visible. Pour the hot solution upon a glass plate and allow to set. With a large cork-borer (diam. 10·15 cm.), cut into discs which are dried, measured and weighed. Seven Petri dishes are required and are almost filled with the following fluids: (i.) water, (ii.) N/10HCl or H_2SO_4, (iii.) N/10NaOH, (iv.) N/2KI, (v.) N/2NH_4CNS, (vi.) N/5$CaCl_2$, (vii.) Sat. $MgSO_4$.

Put a few gelatin discs (not touching one another) into each dish, immersing them quickly and completely to avoid the adherence of air bubbles. After an hour's immersion some of the discs will have visibly swelled. Leave for 24 hours and examine against a black background. Measure and weigh. Which disc has swelled most? Arrange the electrolytes in a descending order as they have favoured imbibition. This is the so-called *lytropic series.* Instead of gelatin, 1 per cent. agar may be used. The lytropic series will be in the same order (see also Experiment 40).

(*g*) *Effect of Acid on Imbibition.* (i.) Stretch a piece of catgut vertically between a weight and a weighted frog-heart lever so that weight and catgut lie in a tall cylinder. The lever may be made to mark a smoked rotating drum. Set the drum going very slowly and almost fill the cylinder with water. Note the changes. Now add sufficient hydrochloric acid to make the whole fluid 1–2 per cent. acid and note the result.

(ii.) Prepare a series of tubes covering a wide range of acidity and alkalinity, *e.g.* HCl approximately 5 per cent., 2·5 per cent., 1·25 per cent., 0·6 per cent. and 0·2 per cent., and a similar range for Na_2CO_3. Place a weighed piece of blood fibrin in each and in a tube of water. After half an hour remove the fibrin, dry with blotting paper and weigh. Fibrin absorbs more water in any solution of acid or alkali than in pure water (cf. Experiment 42 (*c*)).

(iii.) Remove the eyes from a dead experimental animal or get sheep's eyes from the abattoir. Measure the diameters of each eye. Weigh the eyes. Place one eye in one of each of the following solutions, 0·5 per cent. HCl, 0·5 per cent. HCl + 3/20 M . $Ca(NO_3)_2$, 0·5 per cent. HCl + N/10 NaCl, 0·5 per cent. HCl + N/10 Na_2SO_4. Leave for 6–10 hours and then dry with blotting paper, measure and weigh. The eyes in the pure diluted acid swell very rapidly. The presence of salts prevents much swelling and, in the case of sodium sulphate, may even cause a decrease in weight. Similar results may be obtained with pure gels placed in collodion bags.

40. **Gelation.**

(*a*) Heat a small quantity of 1 per cent. solution of (1) gelatin, (2) serum, (3) dextrine. Cool. What is the result? Has reheating any effect?

(*b*) *Effects of solutes on gelation.* Into four boiling tubes put the same quantity of 2½ per cent. gelatin. In one tube dissolve about 5–7 per cent. magnesium sulphate crystals. Potassium iodide crystals are added to the second tube, while a few drops of 40 per cent. formalin are mixed with the gelatin in the third tube. The fourth tube is left as a control. Allow all tubes to stand overnight and examine by tilting and shaking. How do you explain the varied viscosity?

Sulphates, citrates and phosphates increase the viscosity of aqueous emulsoid gels. Iodides, bromides, cyanides and some chlorides similarly decrease viscosity. Alcohol, formaldehyde, etc., in small amounts increase viscosity.

(*c*) *Effect of non-electrolytes on the setting of gelatin.* A 6 per cent. solution of gelatin is prepared and divided into three exactly similar tubes. To one tube is added enough cane sugar to make a 10 per cent. solution. To another sample sufficient urea to make a 5 per cent. solution. The third tube will contain gelatin alone. Warm all tubes in a water bath to ensure complete liquidity and uniformity. Put 1 c.c. of fluid from each tube on to three separate watch-glasses and add a small lead shot to each, cover with a second watch-glass to prevent evaporation and gently rock the glasses from time to time and note how long the fluid in each takes to set (lack of mobility of shot).

Examine the firmness of the gelatin when set.

When the gelatin left in the tubes has set, turn out the little cylinders of gel. Weigh, and measure each diameter.

Place them in a relatively large volume of water overnight. Dry with blotting paper and again weigh and measure.

Sugar causes the gel to set more rapidly and swell less than either of the other two. Urea retards gelation and causes the largest imbibition of water.

Similar experiments may be made with other non-electolytes, *e.g.*:

1. Control . .	6 c.c. of 6 per cent. gelatin	+ 1 c.c. of water.
2. Urea . .	,, ,,	+ 1 gram of urea.
3. Aldehyde .	,, ,,	+ 1 c.c. 40 per cent. formalin.
4. Alcohol .	,, ,,	+ 1 c.c. methylated spirit.
5. Cane Sugar .	,, ,,	+ 1 gram sucrose.

(*d*) *Effect of electrolytes on gelation.* (i.) Effect of various anions.

1. Control tube .	3 c.c. 6 per cent. gelation	+ 3 c.c. water (very faintly acid).
2. Sulphate . .	,, ,,	+ 3 c.c. normal K_2SO_4.
3. Sulphocyanide	,, ,,	+ 3 c.c. N. K.CNS.
4. Chloride .	,, ,,	+ 3 c.c. N. K.Cl.
5. Salicylate .	,, ,,	+ 3 c.c. N. $K.C_7H_5O_3$.

(ii.) Effect of various cations.

1. Control tube .	3 c.c. 6 per cent. gelatin	+ 3 c.c. faintly acid water.
2. Sodium . .	,, ,,	+ 3 c.c. N. Na_2SO_4.
3. Calcium . .	,, ,,	+ 3 c.c. 0·03 N. $CaSO_4$. (sat. sol.)
4. Magnesium .	,, ,,	+ 3 c.c. N. $MgSO_4$.

5. Potassium .	3 c.c. 6 per cent. gelatin	+ 3 c.c. N. K_2SO_4.
6. Ammonium .	,, ,,	+ 3 c.c. N $(NH_4)_2 SO_4$.
7. Iron . .	,, ,,	+ 3 c.c. N. $Fe_2(SO_4)_3$.

41. **Study of Syneresis.**

(*a*) Make up about 30 c.c. each of 3 per cent., 1·5 per cent., and 0·75 per cent. gelatin. Add a drop of thymol in chloroform to each sol, and place in stoppered weighing bottles for three or four days. Note contraction of the gel with expression of clear fluid. Examine both gel and fluid in each case and note that qualitatively they are alike. That is, the separated phases contain gelatin and salts dispersed through water and the gels, water and salts dispersed through gelatin.

(*b*) A similar series of experiments may be performed with 4 per cent. and 2 per cent. starch.

(*c*) Examine the curd and the whey produced (*a*) by the addition of essence of rennet, or (*b*) by a drop of acid to 20 c.c. of warm (30–38° C.) milk.

(*d*) Put about 5 c.c. of freshly drawn blood into each of a pair of centrifuge tubes and spin gently for about 10 minutes. Remove from centrifuge, add a drop of preservative, cork firmly and allow to stand overnight. Draw off the clear fluid (serum) and test for proteins and chlorides. Examine the clot. Note clear layer on top—the buffy coat. Cut this part away and test it for proteins and chlorides. Put the lower red portion in a fold of muslin and knead it in a little 0·9 per cent. saline in a small evaporating basin. Note (1) emergence of red corpuscles, (2) residue of tough fibrin. Treat the remainder of the buffy coat in the same way and note the fibrin residue.

42. **Emulsions.**

(*a*) (i.) Take four test tubes and place in each 10 c.c. of olive oil. In addition add to (α) a few drops of oleic acid and a drop of alcoholic NaOH ; to (β) some (soft) soap solution ; to (γ) a few drops of oleic acid and about the same quantity of conc. $Ca(OH)_2$ solution, shake and allow to stand. Which give the best emulsions ?

(ii.) *Another method of preparation.* Place some gum acacia in a large mortar. Powder it thoroughly. While continuing the rotatory movement of the pestle, add the oil to be emulsified in a very slow stream. Keep the pestle going, *always in the same direction*. After a thorough mixture has been produced add sufficient water to emulsify the gum, starting with a few cubic centimetres and gradually increasing the rate at which the water is run in. Keep the pestle going. When the emulsion gives forth a cracking sound, the rest of the water may be added in one lot.

(*b*) *To determine the optimum concentration of colloid for the stabilisation of an emulsion.* Into each of three mortars introduce 20 c.c. of water, (α) containing 1·25 per cent. of commercial soft soap, (β) 1·875 per cent., and (γ) 2·5 per cent. To each slowly add 120 c.c. of, say, cottonseed oil, stirring regularly but not too vigorously meanwhile. If possible, put on a mechanical shaker for half an hour. Pour into tall cylinders and allow to stand for some days.

(*c*) *To determine the effect of the* pH *of the colloid on the stability of the emulsion.* To 5 gm. of dry casein in each of three mortars add slowly (α) 50 c.c. N/20NaOH, (β) 50 c.c. water, and to (γ) 50 c.c. N/20HCl. Allow to stand overnight and then slowly stir 75 c.c. of cottonseed oil into each. Pour into clean jars and allow to stand. Why does (β) separate out ?

(*d*) *Effect of concentration of oil on rigidity.* Stir into four lots of 25 c.c. of 25 per cent. soft soap in mortars, (α) 50 c.c., (β) 100 c.c., (γ) 200 c.c.,

(δ) 400–500 c.c. of cottonseed oil. Place in shallow dishes. Note rigidity. What happens when the optimum concentration of oil has been passed.

(*e*) Divide an emulsion of oil in water—(soap), (*i.e.* 120 c.c. oil in 20 c.c. 7 per cent. soft soap) into nine portions. No. (i.) will serve as control. To the others add a few drops of one of the following N solutions, (ii.) HCl, (iii.) NaOH, (iv.) $Ca(OH)_2$, (v.) $CaCl_2$, (vi.) NaCl, (vii.) Alcohol, (viii.) $CHCl_3$, (ix.) Ether.

Instead of soap any hydrophilic colloid may be used, *e.g.* albumin, casein, acacia, dextrin, starch. The carbohydrate-stabilised emulsions are the hardest to break.

43. **Foams.**

(*a*) Place about 10 c.c. of distilled water, absolute alcohol, and glacial acetic acid in separate test tubes and shake vigorously for two minutes. Does a foam appear? Now mix 5 c.c. of water with 5 c.c. of the alcohol; and also with 5 c.c. of the acetic acid; and the remaining 5 c.c. of the alcohol with 5 c.c. of the acetic acid. Again shake vigorously. (*Caution.*—Release pressure occasionally.) Do foams appear? How long do they last? Dust a little lycopodium powder on to the aqueous alcohol, and a little finely powdered lamp-black on to the surface of the aqueous acetic acid. Again shake for two minutes. The foams last much longer.

(*b*) Shake up some protein sol, *e.g.* diluted blood serum, 1 per cent. egg albumin, or 1 per cent. Witte's peptone in 0·5 per cent. NaCl. Touch the froth with a glass rod on which is a drop of either olive oil, caprylic acid or cheese. Why does the froth subside.

(*c*) Put 5 c.c. of a rennin solution into each of three test tubes. Leave tube 1 as control. Add a trace of saponin to tube 3. Shake tubes 2 and 3 vigorously for two minutes. Withdraw 2 c.c. from each tube and compare their activity in curdling a calcified milk (see Experiment 47). Why has the saponin prevented the inactivation of the enzyme produced by shaking.

44. **Conditions Governing Enzyme Action.**

Collect 10–20 c.c. saliva, filter.

(1) Optimum temperature for the action of ptyalin on starch. Measure 5 c.c. of 1 per cent. boiled starch solution into a test tube and add 1 c.c. of saliva. Set up the tubes at the following temperatures, (*a*) 0° C., (*b*) 20° C., (*c*) 40° C., (*d*) 60° C., (*e*) 100° C. Test every minute with dilute iodine solution until the achromic point is reached. Note the time taken.

(2) Optimum *p*H. One cubic centimetre of 1 per cent. starch solution, 5 c.c. of a buffer solution, 1 c.c. of 0·9 per cent. NaCl solution, 1 c.c. saliva and 4 c.c. distilled water are measured into a test tube, the tube is set in a water bath at 37° C. Note the time taken to reach the achromic point. Buffer solutions of the following *p*H values to be used: (*a*) 8, (*b*) 7·4, (*c*) 6·8, (*d*) 5·8, (*e*) 4·8.

(3) The action of salts in enzyme action. (*a*) Take 1 c.c. of starch solution, 1 c.c. of distilled water, and 1 c.c. of dialysed saliva.

(*b*) Take 1 c.c. of starch solution, 1 c.c. of 0·9 per cent. NaCl solution, and 1 c.c. of dialysed saliva.

Set the tubes in a water bath at 37° C., and note the time taken to reach the achromic point.

(4) Effect of boiling on saliva. To 1 c.c. of starch solution add 1 c.c. of boiled saliva. Does the saliva have any action when placed at 37° C.?

(5) Effect of boiling on starch. Add a little raw starch to 10 c.c. of water. Divide into two equal portions, boil (*a*) and allow to cool, then add 1 c.c. of saliva to each portion and place the tubes in a water bath at 37° C. Note the time taken to reach the achromic point.

45. The Influence of the Hydrogen Ion Concentration on the Activity of an Enzyme. Ptyalin (adapted from Ringer, *Zeits. f. physiol. Chem.*, 1910).

Material required. (1) About 50 c.c. of a 1 in 50 dilution of saliva. (2) Seven large boiling tubes or small Erlenmeyer flasks of about 50–100 c.c. capacity. The tubes should contain phosphate buffers made up as on p. 525, so that each tube has 5 c.c. of a phosphate solution with the following *p*H values: 6·24, 6·47, 6·64, 6·81, 6·98, 7·17, 7·38. (3) 0·5 per cent. solution of boiled starch made up in 0·3 per cent. sodium chloride solution. (Ptyalin operates best in the presence of the Cl ion.) (4) A series of about 30 small tubes, each containing 5 c.c. of an approximately N/1,000 iodine solution to act as indicators.

Method. To each boiling tube in turn add 5 c.c. of the diluted saliva and mix. Starting from the left *and at an interval of exactly two minutes* between each flask, add 25 c.c. (or 50 c.c. if your tubes will contain it) of the starch sol. Then at two-minute intervals, 2 c.c. of tube 3 are transferred to an iodine tube. At first the colour will be blue, later violet, later still red. At this stage, *without delay*, remove in turn 5 c.c. of the contents of each tube and transfer to separate iodine tubes. *Note that as before, exactly two minutes should* elapse between the withdrawal of the reaction mixture from successive tubes. The following is a typical result:

Tube No.	1	2	3	4	5	6	7
*p*H (approx.) . .	6·3	6·5	6·7	6·8	7·0	7·2	7·4
Iodine colour . .	blue	violet	red	yellow	red	red violet	violet

In tube 4 the reaction has proceeded most rapidly, *i.e.* *p*H 6·8 (approx.) is the optimum *p*H for ptyalin.

46. Effect of Removal of the End-products on the Rate of Action of Ptyalin (p. 125).

Place a small quantity of saliva in a test tube and dilute with an equal volume of water. Divide this amount equally between a dialysing cylinder *A* and a slide-tube *B* of approximately the same diameter. Add an equal quantity of 0·5 per cent. boiled starch (in 0·3 per cent. NaCl) to each tube and mix the contents. Place the tubes in a beaker and maintain a constant flow of water in the beaker. The flow of water will maintain a comparatively steady temperature in both tubes and will hasten dialysis in tube *A*. By means of a glass rod (one to be kept for each tube) transfer, from time to time, a drop from each solution to a white glazed tile and add to each drop a little iodine solution. In the drops from tube *A* a blue colour with iodine is given at first; later drops give a purplish, later still a reddish brown colour, and after about an hour no reaction with iodine is obtained. At this stage the drops from tube *B* still give a clear indication of the presence of starch. A typical result is given in Table XXI. on p. 125.

A suitable exercise is now to estimate the amount of reducing sugar present in each tube.

47. Estimation of the Relative Activity of an Enzyme (Rennin).

Material required. (i.) *Boiled* milk, to which has been added one-tenth of its volume of 10 per cent. calcium chloride solution. (ii.) Arbitrary standard of activity—prepared by diluting either commercial "Essence of Rennet" or Benger's "Liquor Pepticus" to such a strength as will produce the curdling of an equal volume of $CaCl_2$ milk mixture in about 10–12 minutes *at room*

temperature. (iii.) Filtered gastric juice or an artificial gastric juice made from *pepsin porci* or from one of the above-mentioned commercial extracts.

Method. Take nine test tubes and put 1 c.c. of water into each except the first. Into the first two tubes put 1 c.c. of the juice to be examined. Mix the contents of the second tube by repeated sucking up into the pipette and blowing out. Transfer 1 c.c. of the mixture to the third tube, from which, after similar mixing, remove 1 c.c. and put it into the next tube, and so on, rejecting the 1 c.c. removed from the ninth tube. The tubes now contain 1 c.c. of fluid consisting of the juice of unknown strength diluted as follows :

Tube No.	1	2	3	4	5	6	7	8	9
Dilution .	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256

Place the tubes in a rack with a space between tubes 5 and 6. In this space put a tube containing 1 c.c. of the prepared standard juice. To each in turn add 1 c.c. of the calcium-chloride-milk mixture. This must be done as rapidly as possible. Use a graduated 10 c.c. pipette for the purpose. The operation should then take less than a minute to perform. Hold the rack of tubes in the hand and gently tilt it occasionally, observing the way in which the milk mixture flows on the sides of the tubes. Determine which tubes show curd formation simultaneously with the standard tube. Suppose the fourth tube curds a little before the "standard," which curds a little before tube 5. The unknown juice, therefore, is between eight and sixteen times as strong in rennin action as the standard.

A new series of dilutions should now be made. Dilute the unknown juice eight times, and with a graduated 2 c.c. pipette put the following amounts of juice and water into six tubes.

Tube No.	1	2	3	4	5	6
Diluted juice (c.c.) .	2	1·6	1·3	1	0·7	0·4
Water (c.c.) . . .	0	0·4	0·7	1	1·3	1·6

A control tube containing 2 c.c. of the standard enzyme solution is placed near the middle of the series ; 2 c.c. of the milk mixture is added as before and the above procedure carried out. Suppose now the control tube and tube 3 almost coincide in clotting time. If tube 3 is just a little earlier in clotting than the control, take an interpolated value, 1·2. Then 1·2 c.c. of a 1/8 solution of the unknown has an "enzyme strength" = 2 c.c. of the standard strength. That is, the unknown juice contains 8 × 2/1·2 times as much enzyme as the standard = 13·3.

Similar experiments may be carried out with other enzymes, using suitable substrates. For example, determine the "strength" of the ptyalin in your own saliva, using a diluted solution of Taka diastase as standard.

In determining the strength of proteolytic and lipolytic enzymes a water bath capable of containing the labelled tubes and of being maintained at a constant temperature (38–40° C.) is essential. For substrate for the proteolytic enzymes either the coagulated egg albumin suspension or the turbid suspension produced by the precipitation of the serum proteins by sulpho-

salicylic acid may be used (p. 562). It is necessary, of course, to maintain the correct pH for the particular enzyme.

48. **Demonstration of the Presence of a Lipase in an Extract.** (Benger's Liquor Pancreaticus.)

Take 90 c.c. of distilled water, 10 c.c. of M/3 secondary sodium phosphate and 20 drops of tributyrin, and shake together for 10 minutes. Filter, rejecting the first few cubic centimetres of the filtrate. This gives a fine suspension of fat in a buffered solution of about pH 8. To 50 c.c. of this mixture at 38° C. add 2 c.c. of Benger's Liquor Pancreaticus. Mix and rapidly withdraw about 5 c.c. for stalagmometric investigation (Experiment 17 (1)). The drop number so obtained is taken as that of a diluted tributyrin mixture. If a pancreatic lipase is present in the liquid under test, it should cause the splitting of the fat into butyric acid and glycerol, which mixture has a higher surface tension than the parent substance, and so gives fewer drops per 3 c.c. The mixture is kept at about 38° C., and lots of 5 c.c. are removed, *cooled*, and the drop number taken every 5-10 minutes, depending on the activity of the lipase.

Time (minutes)	0	5	15	25	35
Drop No.	120	120	113	109	101

49. **Estimation of the Relative Lipolytic Activity of an Extract of Pancreas.**

Prepare a series of test tubes with 2 c.c. of the following dilutions of 1/20 Benger's Liquor Pancreaticus: 1/1, 1/2, 1/4, 1/8, 1/16, 1/32. Place a seventh tube about the middle of the series and put in it 2 c.c. of the extract of pancreas. Now add as rapidly as possible to each tube in order 5 c.c. of the phosphate-tributyrin mixture used in the previous experiment. Immerse all the tubes in a water bath at 38° C. for 30 minutes. Cool. Estimate the relative surface tension of the mixtures by the capillary rise method (Experiment 17 (2)), starting at the right, *i.e.* with the greater dilutions of the enzyme. Suppose the unknown fluid rose in the tube just a little less than in tube 3 but a little more than in tube 4, then the pancreatic extract would approximately be in strength between $1/4 \times 1/20 = 1/80$ and 1/160 of the liquor pancreaticus. One may then proceed as in the experiment above to define the strength more accurately.

50. **Chemical Gardens.**

(*a*) Place 50 c.c. of potassium ferrocyanide in a glass jar or beaker and add a small particle of ferric chloride (small pea). A semipermeable membrane of ferric ferrocyanide (Prussian blue) is formed round the solid. Endosmosis occurs and peculiar growths may be formed.

(*b*) Add a drop of almost saturated potassium ferrocyanide from the end of a glass rod to a solution of copper sulphate (bench reagent). A semipermeable membrane of copper ferrocyanide is formed round the drop and endosmosis takes place. This causes an increase in the concentration of the copper sulphate immediately round the drop and blue "rootlets" may be seen descending from the drop. These are due to the increased density of the sulphate (see also Experiment 5 (*a*)).

51. **Leduc's Growths.**

A small flat-sided jar, *e.g.* a specimen jar, is filled with a 1-2 per cent. solution of gelatin to which is added just enough potassium ferrocyanide to give it a pale green colour. Just before the gelatin has set, a little seed

made from a mixture of glucose and copper sulphate is planted on the bottom of the jar. Within an hour, growth will be visible and may proceed for several days.

See Leduc, *Études de Biophysique*, I. *Théorie Physico-Chimique de la Vie* (1910); II. *La Biologie Synthétique* (1912).

52. **Shell Formation** (Rhumbler).

(1) Mix a little powdered glass with chloroform and set a drop of the mixture in water. The glass particles gather rapidly round the surface of the drop.

(2) Repeat the experiment, using fine silver sand dispersed through oil and finally dropped into 70 per cent. alcohol. The movements take place more slowly and the drop requires about three hours to attain equilibrium.

53. **Study of Living Cells.**

(*a*) *Amœba.* The large form may be found in water trickling from a boggy spot. Collect some of the upper layers of ooze from the bottom of the ditch or boggy runlet and leave for a few days in tall jars to allow the ooze to settle. Pipette off the surface layer and transfer to a test tube containing clear pond water with some green algæ in it. The amœbæ will be found on the surface of the ooze.

Small amœbæ may readily be obtained from garden soil by the following method (Goodey, *Nature*, 1918). Boil some hay or grass in water. Filter. Neutralise filtrate. Take 2–3 gm. of garden earth in each of several Petri dishes and mix with the filtrate from the hay infusion to give a depth of about 2–3 mm. of moist soil. Place in a good light and *keep moist.* After 2–3 days float some cover-slips on the surface of the fluid in the dishes. The amœbæ will attach themselves to the under surface of the slips. Remove slips and rinse *gently* in water in an evaporating basin. Mount in a hanging drop slide with a hair or thread placed below the slip to aid in focussing.

Examine the slide. Select as large an amœba as possible and make drawings of it from minute to minute. Place a small drop of N/10 HCl at one side of the preparation and note what happens. Now place 2 drops of N/10 NaOH on the same spot and observe any changes.

Electrical stimulation. Use a slide with electrodes (Fig. 20) and attach the electrodes *through a short-circuiting* key to the secondary coil of an induction apparatus. Pull the coil well out, start the interrupter, and open the short-circuiting key for a moment. If no change occurs, gradually push in the coil and try again. Do not expose the amœba to too strong or too long a shock or it will be disintegrated.

(*b*) *Blood corpuscles.* (i.) Take three test tubes and place in one about 5 c.c. of water and in another a similar amount of 0·9 per cent. sodium chloride, and in the third 2 per cent. sodium chloride. Prick the finger with a sterile needle and add the same number of drops of blood to each tube. Shake and examine the tubes (α) as to opacity and (β) as to depth of colour. Take a drop of the fluid from each and examine under the microscope. Measure the diameter of a number of corpuscles and average those from each tube.

(ii.) Add a drop of fresh blood to a drop of 0·5 per cent. sodium chloride solution on a microscope slide. Place a card on the side of the microscope stage and keeping both eyes open trace the projection of a corpuscle from time to time or measure the diameter.

(iii.) Experiments similar to those detailed above for *amœba* may be performed on the leucocytes.

54. Conditions Affecting Growth, etc.

To a test tube *filled* with a nutrient medium add 1 drop of yeast emulsion. (Nutrient medium contains the elements C, O, H, N, S, and P, *e.g.* Urea $CO(NH_2)_2$, glucose $C_6H_{12}O_6$, with traces of Na_2HPO_4, and $Ca_3(PO_4)_2$.) Add a similar drop to a test tube filled with distilled water. Shake the tubes well and examine a drop of the mixture from each microscopically, counting the number of cells in three fields; take the average. Note the appearance of the mixtures.

Student at bench (1). Insert a cork fitted with a glass tube into the test tubes, and place them in the incubator at 37° C.

(2) As in (1), placing the tubes in ice.

(3) Introduce a few drops of pure phenol into each of the tubes, insert the corks as in (1) and place the tubes in the incubator.

(4) Boil the mixtures, cool the tubes under the tap, insert the corks and place the tubes in the incubator.

The tubes are left under these conditions for 24 hours.

Results. Examine the tubes before and after shaking. A drop from each tube is to be examined microscopically after shaking, counting the number of cells in three fields; take the average.

Record the results on the sheet provided, using the signs + and −.

	Gas	Opacity.	Number.
Tubes with sugar (1) at 37° C.			
Tubes with sugar (2) at 0° C.			
Tubes with sugar (3) with phenol			
Tubes with sugar (4) boiled .			
Tubes with water (5) at 37° C.			

Test (1) is also carried out in bulk, the yeast cells are removed by filtration. Use some of the filtrate for the following tests.

(*a*) Disappearance of sugar. To about ½ in. of the fluid in a test tube add an equal volume of Folin's copper solution and boil for one minute. Note the extent of reduction. Repeat, using the original nutrient medium. Compare the reduction obtained with that obtained in the preceding test.

(*b*) Formation of alcohol. Some of the filtrate from (1) is distilled. Test the distillate for alcohol. To about ½ in. of the distillate add a few drops of Pot. bichromate and a little H_2SO_4 conc., warm.

Note. (1) Pungent odour, aldehyde. (2) Green colour.

Nature of the gas evolved. Fill a test tube with the nutrient medium, and, covering the mouth of the tube with a coin, invert it into a dish containing nutrient medium; care must be taken to avoid entrance of air bubbles into the tube. Introduce a piece of yeast about the size of a pea so that it comes to lie in the mouth of the inverted test tube, which is now allowed to rest on the dish. Support the tube by means of a burette stand and place in the incubator at 37° C. for 24 hours.

With the mouth of the tube still beneath the surface of the fluid in the dish, introduce into the tube a few cubic centimetres of 40 per cent. NaOH using a bent pipette.

Conclusions. (1) What has happened to the yeast cells in each of the tubes?

(2) What conclusions do you come to as to the influence upon the yeast protoplasm of the various conditions to which it has been subjected?

(3) Where does the yeast protoplasm get material for growth ?

(4) Where does yeast protoplasm get the energy for growth ?

55. **Mimicry of Cell Structure** (Herrera after Harting).

A crystallising dish 18 cm. in diameter is filled with colloidal silica. This may readily be prepared by dissolving freshly precipitated gelatinous silica in a solution of ammonia (density 26°). Silica is added till all the ammonia has been driven off and the colloid has a density of over 1·032 (*i.e.* 3–5 per cent. solid silica). (A solution of sodium silicate of a density of 1·020 may be used instead of colloidal silica.) At one edge of the crystallising dish place 10–20 mgrm. of recrystallised potassium bifluoride. At the diametrically opposite side of the dish place 5 gm. pure powdered anhydrous calcium chloride. Cover and keep at 20° C. for 24 hours. Various structures which may be stained by any of the dyes used by histologists may be seen, *e.g.* nucleated amœbæ, cells undergoing division, nuclear spiremes, granular and honeycomb structures, etc.

The figures are due to the strains produced in the silicate by the simultaneous formation of a colloid, calcium silicate and a crystalloid, calcium fluoride. Silica, coagulated by a crystalloid, gives rise to a semipermeable membrane, which, if it forms a sac round a crystalloid, may set up endosmosis. Slow amœboid movements may be shown by some of the complexes lying near the point of insertion of the $CaCl_2$. Add a trace of alcohol over the $CaCl_2$, and more rapid diffusion ensues.

56. **Action of Ultra-violet Light.**

The light used is passed through a Wood's filter which cuts off all the visible rays. *Caution must, therefore, be exercised to prevent any of the direct rays from entering the eye.* If an adequately screened lamp like the K.B.B. microscope lamp is used, goggles need not be worn. With unscreened lamps they are essential.

(*a*) *To render the rays visible.* Thin glass test tubes may be used, but better results are obtained with flat-sided quartz vessels. Place vessels containing solutions of fluorescent substances in the rays and about a foot away from the Wood's filter. Try quinine, eosin, fluorescein, and dilute hæmatoporphyrin.

It is interesting to note the beautiful fluorescence obtained with " natural " pearls and the absolute lack of it with imitations. " Cultured " pearls vary. They all fluoresce, and some of them are almost as fluorescent as the " natural" pearl.

(*b*) *Bleaching effect.* Expose the following solutions for one minute at 1 ft. from the lamp. Acetone-methylene blue, 20 per cent. potassium ferrocyanide, carbon-tetrachloride + potassium iodide. Rays below 2,650 A° cause the liberation of nascent chlorine from the tetrachloride. The chlorine replaces the iodine in the KI. The iodine thus set free gives the tetrachloride a reddish violet tinge.

Filter paper if soaked in potassium ferrocyanide is bleached, and if soaked in paraphenylenediamene nitrate turns violet on exposure to the short rays.

(*c*) *Schanz' experiment.* A dilute solution of egg albumin exposed for an hour in a quartz vessel close to the lamp (within 5 cm.) is so changed in colloidal state that it acts to half-saturation with ammonium sulphate like a globulin, *i.e.* it tends to lose its emulsoid character and become more like a suspensoid. It ceases to protect gold (Experiment 37).

(*d*) *Effect on enzymes.* Expose an active solution of any of the digestive enzymes for an hour near the lamp and compare their activity with that of some of the unexposed solution. Maltase reacts very rapidly.

(*e*) *Effect on Cyclops quadricornis.* Place one or two of these pond crustaceans in two Petri dishes containing the minimum amount of tap water. Cover one dish with a 26-oz. glass. Note movements. Now place both dishes below the lamp so that they are equally radiated. Note the time. At first mobility in the uncovered dish is greatly increased. Soon, however, movement is gradually slowed down and stops in less than 1–2 minutes. Note time. The cyclops in the covered glass should now receive attention. They live eight or nine times as long as the uncovered ones. Glass, therefore, cuts off some of the abiotic radiations. If vita-glass or other similar glass is available it is instructive to cover a third Petri dish with it.

(*f*) *Effect on living skin.* Cut three holes of various patterns in line on a piece of thick brown paper. Clean a part of the back of the arm with spirit soap and dry with methylated spirit. Fix the brown paper over this part of the arm with rubber bands. Cover hole No. 1 with a thin layer of vaseline; leave No. 2 uncovered, and cover No. 3 with either one layer of muslin or a piece of vita-glass. Expose to the rays at a distance of 1 metre for two minutes. Remove the paper and examine the arm immediately and one hour afterwards. Compare notes next day with the rest of the class. Does the colour of hair and eyes have any influence on the amount of erythema produced?

57. **Indicators of Potential Difference.**

Two of the more common indicators of the existence of a potential difference between two points have been considered in detail, viz. the capillary electrometer (on p. 50, Experiment 21) and the string galvanometer (Chap. XXVI.). If the potential difference is sufficiently great or if it is amplified (one valve resistance-capacity amplifier) it may be demonstrated and measured by an ordinary mirror galvanometer. A wireless headphone or, if two or three valves (resistance-capacity coupled) are used in series, a loudspeaker makes a very efficient detector of differences of E.M.F.

58. **The current of injury,** etc., of muscle (pp. 152 and 179) is usually measured by *compensation.*

A cell of constant known E.M.F. is put in the same circuit as the tissue giving rise to the current, but sending its current in the opposite direction (Fig. 107 and Experiment 23). By moving the jockey along the slide wire (R_1-R_2) till the E.M.F. from the cell exactly balances the demarcation current, *i.e.* till the meniscus at the mercury-acid interface becomes steady, one may determine what relationship the constant E.M.F. bears to the muscle E.M F. Non-polarisable electrodes must be used.

59. **The Membrane Potential of the Skin of an Apple.**

Select an undamaged apple. With a cork cutter remove a small circle of skin from one side. Place the apple whole side downwards in a Petri dish containing some 0·1 N . KCl solution. A non-polarisable electrode is placed in this solution in contact with the apple. The other similar electrode is brought into contact with the upper cut surface of the apple, taking care not to touch any of the skin. Lead these electrodes to a capillary electrometer or to a sensitive mirror galvanometer. The damaged portion is, of course, the negative pole.

60. **Model of Mucoid Secretion.** (Fischer.)

Grind up in a mortar a small quantity of gum acacia and 1 or 2 c.c. of cottonseed oil. Put a drop of this mixture on a microscope slide with cover glass and examine. Place a drop of water at the edge of the cover slip and note what happens when it touches the oil layer.

61. **Model to Illustrate some Phases of Urine Formation.** (Fischer and MacLaughlin.)

Prepare some cups of sodium stearate by pouring the hot stearate solution (1/10 molar) into a mould consisting of one beaker (120 c.c.) suspended within another similarly shaped (350 c.c.). A cup is supported on a filtration disc in a filter funnel whose stem enters the mouth of a graduated cylinder. Another cylinder (gas jar) or bottle inverted may be used as a constant level device. Fill the cup and the gas jar with the solution. Invert the full jar and suspend it vertically so that its mouth just dips below the surface of the fluid in the cup. From time to time measure the fluid in the lower graduated cylinder, say every hour.

Try the following solutions: (*a*) water (3–6 hours), (*b*) molar sodium oleate (24 hours), (*c*) NaCl 1/8 M., 1/4 M., and 1/1 M. (3–6 hours), (*d*) 1/8 M. $CaCl_2$ (1–4 hours), (*e*) 1/8 M. NH_4Cl (7 hours).

Note increased flow with (*c*) and (*d*); initial increase and final decrease of flow with (*e*) and total inhibition with (*b*). Test, in each case, the perfusion fluid, the perfusate and the cup, with phenolphthalein. How do you account for your results? Test the perfusates for fatty acids. Why does pure water dissolve out more of the acid than do the salt solutions?

62. **Specific Gravity of Blood.**

Make up a series of solutions of sodium sulphate or of mixtures of benzene and chloroform to give specific gravities ranging from 1·04 to 1·07. Puncture the finger (use a *sterile* blood lancet, *i.e.* one with a flat sharp edge and not a sewing needle) and allow a drop of blood to fall into each tube of liquid. *See that the drop pierces the surface of the liquid.* Note in which tube the blood drop remains suspended or sinks very slowly. The specific gravity of the fluid in that tube is equal to or almost equal to the specific gravity of the blood.

Preparation of (*a*) *Sodium sulphate solutions.*

Specific gravity . . .	1·04	1·045	1·05	1·055	1·06	1·065	1·07
Grams Na_2SO_4 in 500 c.c. of water	50	55	70	85	90	95	100

(*b*) *Benzene and chloroform mixture.* Make a mixture of chloroform and benzene of specific gravity 1·07. Stir in benzene to get the smaller values of the specific gravity.

Another method. Place the mixture of chloroform and benzene with a specific gravity of 1·07 in a urinometer tube. Drop one drop of blood from the finger into the tube. If the drop floats add benzene and stir with a glass rod. Continue adding benzene and stirring till the drop of blood neither floats nor sinks. The specific gravity of this mixture taken with a hydrometer is the specific gravity of the blood under test.

63. **Hæmolysis** (see also Experiments 53 (*b*) and 64).

(i.) Mix a drop of your blood with a drop of distilled water on an uncovered microscope slide and observe. The corpuscles swell up, burst and liberate hæmoglobin.

(ii.) Repeat, using instead of water some fat solvent like ether, chloroform etc.

(iii.) Repeat, using a capillary active substance in dilute solution, *e.g.* bile salts, saponin, etc.

64. **Estimation of the Fragility of Human Red Blood Corpuscles.**

Twelve clean small test tubes (4 × ⅜ in.) are placed in a row in a rack and numbered from left to right 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15 and 14.

With a pipette drawn to a fine point put into each tube the number of drops of 0·5 per cent. sodium chloride indicated by the figure on the tube. Rinse the pipette thoroughly with distilled water. Using the same pipette, add 1 drop of distilled water to tube 24, 2 drops to tube 23, and so on, till 11 drops are put into tube 14. Mix. That is, there are now 25 drops of fluid in each tube, giving a graduated series of percentages of NaCl. Thus:

Tube No.	25	24	23	22	21	20
Per cent. NaCl .	0·5	0·48	0·46	0·44	0·42	0·4

Tube No.	19	18	17	16	15	14
Per cent. NaCl .	0·38	0·36	0·34	0·32	0·3	0·28

Add 1 drop of blood (0·1 c.c.) to each tube. Allow to stand at room temperature for 1–2 hours. The test is recorded by + signs, + + + representing complete hæmolysis, + + partial and + initial hæmolysis. The dilution in which there is just a slight hæmolysis is noted as the point of initial hæmolysis. Complete hæmolysis is indicated by the absence of intact corpuscles at the bottom of the tube.

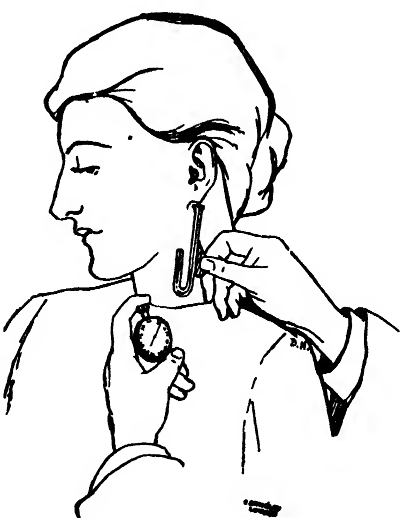


Fig. 110.—Denning-Watson clinical viscosimeter (*Hawksley*).

65. **Determination of the Relative Viscosity of Blood** (see Experiment 31).

Blood may be taken from the lobe of the ear or from the finger. The skin is thoroughly cleansed with ether and pricked with a fine pointed lancet. The viscosimeter (at body temperature) is held vertically under the bleeding spot and the receiver filled (Fig. 110). Measure as in Experiment 31.

Immediately after an observation, the blood should be driven out by a blast from an inflator attached by rubber tubing to the short arm of the capillary.

It is advisable to make a blood count at the same time.

66. **Clotting Time of Blood.**

Thoroughly clean two watch glasses (1½ in. diameter). Clean the lobe of the subject's ear with ether and puncture it with a sterile lancet. Allow a large drop of blood to fall on to one of the watch glasses (as the blood falls set a stop watch going). Add a small lead shot to the blood and cover by inverting the second glass over the first one. Rock the glasses gently and note that, with increasing viscosity of the blood, the extent of movement of the shot is diminished. When the shot ceases to move, stop the watch and note the time taken for the blood to clot. Repeat.

67. Bleeding Time.

Clean the lobe of the ear with ether and puncture with a sterile lancet. As the first drop of blood appears, start a stop watch going. Using the edge of a circle of filter paper, remove the drops of blood as they form. Note the time when no further drop appears.

Repeat. Compare the clotting time and the bleeding time of the same subject.

68. Hæmatocrite.

This apparatus consists of two similar graduated capillary tubes, which, after clipping in a holder, may be spun horizontally by a centrifuge. A small measured quantity of Müller's fluid (Na_2SO_4 – 1 gm.; $K_2Cr_2O_7$ – 2 gm.; distilled water, 100 gm.) is placed in a test glass. The same quantity of blood is added. Mix thoroughly with a glass rod. Fix a short length of rubber tubing furnished with a mouthpiece to each of the graduated tubes and suck up sufficient of the mixture to fill the tubes. Place them in a holder and spin for 5–7 minutes with a velocity of 8,000 revs. per minute. Read off the relative length of the column of corpuscles. As the glass walls of the tubes are thick, it is advisable to aid the eye by looking along a glass plate held at right angles to the tube. The tubes have a bore of one square millimeter and are divided into 100 equal parts. The reading multiplied by 2 will give the volume of corpuscles in 100 parts of blood. The function of the Müller's fluid is to retard clotting and to *fix* the red corpuscles in their natural size.

69. To Demonstrate the Effect of the Tension of CO_2 on the pH of a Solution of Bicarbonate.

Put 5 c.c. of a 0·25 per cent. solution of $NaHCO_3$ in each of three tall stoppered cylinders. To each add 2 drops of neutral red. Fill (*a*) with air expired after a *deep* inspiration, (*b*) with alveolar air (Experiment 70 (*b*)) and (*c*) with CO_2 from a generator or cylinder. Stopper and shake. Note colours. Remove the CO_2 in (*c*) by repeated changes of atmospheric air. Note that the colour goes back from crimson through the red of (*b*) to the orange of (*a*) or even to the yellow seen before any CO_2 was added.

70. Determination of the Alkali Reserve of Blood.

(*a*) *An approximate method.* (Rieger.) *Principle.* Erythrocytes are easily damaged by acid. This will lead to agglutination and hæmolysis on the addition of acid as soon as the reserve of base has been used up.

Method. Ten test tubes (8 in. × 1 in. or thereabout) cleansed thoroughly and dried are set in a rack. The first or stock tube is charged with 9 c.c. of a 0·85 per cent. solution of NaCl (pure salt in distilled water) and 1 c.c. of whole blood (oxalated with 0·2 per cent. pure sodium oxalate). Mix thoroughly by drawing up into the pipette several times, keeping the tip of the pipette always below the surface of the liquid.

One cubic centimetre of the diluted blood is placed in the bottom of each tube, avoiding the sides, and then, starting on the left, N/100HCl is added from a graduated pipette. The first is given 0·75 c.c. acid, the next 0·8 and so on, increasing the amount by 0·05 c.c. with each tube, the last tube thus receiving 1·20 c.c. In about an hour examine the tubes. Those on the left should show no hæmolysis and the corpuscles should be settled in a sharply defined clump in the centre of the foot of the tube. The tube on the right may show hæmolysis and have corpuscles scattered over the bottom in an irregular manner, giving a speckled appearance. The tube with the greatest amount of acid which shows no hæmolysis or scattering of corpuscles gives an indication of the alkali reserve of the blood.

For example: Normal blood—the first six tubes (*i.e.* 0·75–1 c.c.) are clear, tube 7 shows scattering and slight hæmolysis. Therefore 0·1 c.c. of blood can neutralise 1 c.c. of N/100 acid, or 100 c.c. of blood contains base equivalent to 0·84 grams $NaHCO_3$. This would, in Van Slyke's apparatus, give rise to 224 c.c. of "bound" CO_2—a somewhat high result (see p. 334), due probably to interaction of acid and protein and to the buffering action of the oxalate.

In using this method for the determination of the alkali reserve of human blood, the endosmotic effect of the dilution of the blood by the acid may be neglected as the salt concentration does not fall to a value low enough to affect the fragility of the corpuscles. It is advisable, when sheep's or rabbit's blood is used, to make up the acid in 0·75 per cent. sodium chloride. It is also essential to see that, if the blood is not used *immediately it is shed*, that it is kept in an ice chest and is brought to a tension of CO_2 equivalent to alveolar tension. (See Van Slyke's method below.)

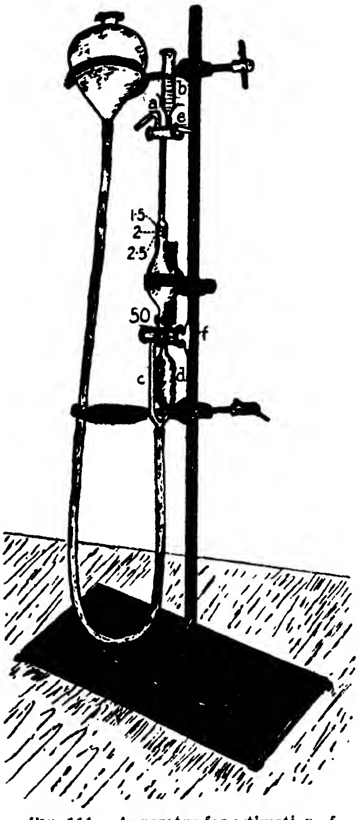


FIG. 111.—Apparatus for estimation of carbonates in solution.

(*b*) *Alkali reserve.* (C. J. Martin.) *Principle.* Dilution of a well-buffered solution such as plasma does not alter its C_H. If an indicator is used which has a low protein error the plasma may be titrated with acid. The titration value indicates the acid-combining power of the plasma.

Apparatus. A small wooden stand to hold three "non-sol" glass test tubes ($8 \times 0·8$ cm.) vertically in a row and close together. The central tube at its upper end runs through the rubber stopper of an inverted "non-sol" flask (100–150 c.c.).

Method. The flask is removed from the central tube and 0·5 c.c. of plasma or serum and 2 c.c. of neutral 0·9 per cent. sodium chloride added. The side tubes are almost filled with a phosphate buffer mixture of $pH = 7·4$. These standard tubes are coloured by the addition of a drop of two aqueous solutions of burnt sugar and flavine (1/100,000) till they match the fluid in the central tube. To all of the tubes are then added 2 drops of 0·05 per cent. (alcoholic) neutral red. The optical effect of the turbidity of the plasma may be counteracted by placing a sheet of white tissue paper behind the tubes. The plasma mixture is titrated with N/50HCl (2 c.c. burette with fine nose) till its colour matches the standards. This is done by running the plasma into the flask, adding a few drops of acid and rotating gently but steadily for 1 minute, the flask meanwhile being in communication with the air. This readily allows the thin film of plasma to give up the liberated CO_2. The fluid is run back into the tube and compared with the standards. The process is repeated as

often as necessary. Rotation for *at least* 1 minute is necessary after each addition of acid.

Titration value for 0·5 c.c. plasma = 0·77 c.c. N/50HCl,
i.e. Alkali reserve of ,, ,, = 0·77 c.c. $N/50NaHCO_3$
,, ,, 100 c.c. ,, = 154 c.c. ,, ,,
= 3·08 N ,,
= 3·08 × 22·4 c.c. CO_2,

i.e. 68·99 volumes per cent. of CO_2 are bound as bicarbonate in the plasma.

A sharper end-point is obtained by the use of phenol sulphonephthalein as indicator. In this case the standard phosphate solutions are made of pH 7·2 to correct the protein error.

(*c*) *Alkali reserve by Van Slyke's method.* The Van Slyke apparatus is illustrated in Fig. 111. It consists essentially of a 50 c.c. pipette with three-way stopcocks (*e* and *f*) at top and bottom, and a 1 c.c. scale on the upper stem, divided into 0·02 c.c. divisions. The body of the apparatus is connected through heavy walled rubber tubing with a levelling bulb filled with mercury. The whole apparatus is supported on a stand so that, without unclamping, the pipette may be rotated round a central axis. The stopcocks are lubricated with a rubber-vaseline mixture and may be held in place by strong rubber bands.

Preliminary preparation. Open taps *e* and *f* and fill the entire apparatus with mercury by raising the levelling bulb, allowing some mercury to run into

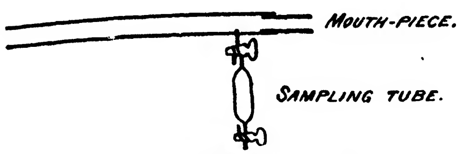


FIG. 112.—Alveolar air collecting tube.

a and into *b*. Shut *e*, and lower the levelling bulb till the mercury falls half-way down *c* and *d*. The bulb is then slowly raised. If the apparatus is gas-free, a sharp click will be heard when the mercury strikes the upper stopcock. If a gas cushion is present, open *e*, and force the gas out, and repeat the evacuation process, opening *f* alternately to *c* and *d*.

Determination. (1) Solutions required. It is convenient to have four dropping bottles with ground in pipettes and rubber teats containing (*a*) 5 per cent. H_2SO_4, (*b*) 1 per cent. carbonate-free NH_3 water, (*c*) caprylic alcohol and (*d*) distilled water. The carbonate-free ammonia is prepared by adding a small amount of sat. barium hydrate solution to ordinary ammonia solution. The barium carbonate is filtered off, and the excess of barium remaining is precipitated with a little $(NH_4)_2SO_4$.

(2) Plasma. An ordinary centrifuge tube is fitted out with rubber cork and glass tubes just like a wash-bottle. The longer tube bears at its upper end a hypodermic needle. The whole apparatus—glass, tubes and needle—is washed out with a saturated solution of neutral potassium oxalate. (Van Slyke and Cullen point out that it is desirable that the subject should avoid vigorous muscular exertion for at least an hour before the blood is drawn. It is also best to avoid stasis, or when stasis is unavoidable the ligature should be released as soon as the vein is entered. In this case, the first sample of blood should be neglected.) The blood should run into the tube without suction. By a gentle rotary motion, mix the blood with the finely crystallised oxalate left adhering to the walls of the vessel, and centrifuge at once. The

plasma is then transferred to sampling tubes (Fig. 112), 3–4 c.c. of plasma to each tube. These tubes (300 c.c.), or separating funnels of the same capacity, are filled with alveolar air (of the subject, if possible). This may be done by holding the tube horizontally, opening both taps and, without inspiring more deeply than normal, expire as quickly and as completely as possible through the tube, closing the further tap just before the expiration is finished. A bottle containing large glass beads must be interposed between mouth and funnel in order to prevent dilution of the plasma by condensation of vapour from the breath. With both taps closed, the funnel is rotated (not shaken) so that the plasma forms a thin layer over the walls, and so readily comes into equilibrium as regards CO_2 tension with the alveolar air.

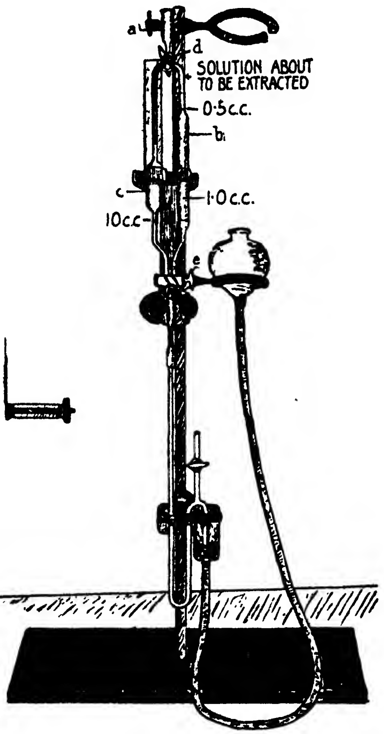


FIG. 113.—Van Slyke micro apparatus.

(3) Procedure. The apparatus is entirely filled with mercury, including the two capillaries (*a* and stem of *b*) at the top. The cup *b* is washed with CO_2-free NH_3. 1 c.c. of plasma is run into the cup from an Ostwald-Folin pipette, keeping the tip of the pipette immersed in the fluid. Placing the mercury reservoir in the second ring (Fig. 111) and with cock *f* open to *d*, open *e* and admit the plasma to the pipette, leaving sufficient in *b* to fill the capillary. Wash the cup twice into the pipette, using about 0·5 c.c. of water each time, adding a very small quantity of caprylic acid to the second wash water. Finally run in 0·5 c.c. of 5 per cent. H_2SO_4 and seal the capillary with mercury. The fluid must come to the 2·5 c.c. mark. Wash out the cup with water and then with carbonate-free ammonia till acid-free. The mercury bulb is now taken to a position (about 80 cm. below the second ring) so that a Torricellian vacuum is formed in the gas pipette. Allow the mercury to run down almost to (but not below) the 50 c.c. mark. Close *f* and replace the bulb on the upper ring. Slacken the milled head of the screw that controls the central swivel. Holding the pipette at the bulb with the right hand and gathering up the loose rubber tubing with the left, rotate the bulb through 180° some 15 times. Set vertically and tighten the milled head. Lower reservoir and, with *f* open to *d*, rapidly empty the water solution into *d* without however allowing any of the gas to follow it. Now open *f* to *c* and, by raising the reservoir, fill the body of the pipette with clean mercury. Hold the reservoir in such a position that pressure inside the reservoir is atmospheric and rapidly take a reading. If thought desirable, re-extraction may be carried out.

Cleaning. Lower the reservoir and run *most* of the mercury back through *c*. Open *f* to *d* and, by raising the reservoir, run the water back into the pipette.

Open *e* to *a* and force the fluid out into a collecting jar. The apparatus is now ready for another determination.

(*d*) *Micro apparatus* (Fig. 113). This apparatus is easier to manipulate, and as water, etc., never enters the gas pipette, it is easily kept clean. The different parts are in the same relative proportions as the corresponding parts of the larger apparatus. Each division of 0·002 c.c. on the smaller corresponds to 0·01 c.c. on the larger.

(1) It is advisable to mark the course of the canals with pencil on the butt end of the tap *d*.

(2) No froth preventer is necessary or advisable. It merely acts on the tap lubricant.

(3) All quantities are reduced to 1/5 of those given above, *e.g.* 0·2 c.c. plasma, 0·1 c.c. water, and 0·1 c.c. acid. In all, exactly 0·5 c.c. of fluid is used.

Calculation of Results.

TABLE FOR CALCULATION OF CO_2-COMBINING POWER OF PLASMA (from Van Slyke and Cullen).

Observed Vol. $\times \frac{B}{760}$.	C.c. of CO_2. Reduced to N.T.P. bound as Bicarbonate by 100 c.c. of plasma.	
	15° C.	20° C.
0·2	9·1	9·9
0·3	18·8	19·5
0·4	28·4	29·0
0·5	38·1	38·5
0·6	47·6	48·1
0·7	57·4	57·6
0·8	67·1	67·2
0·9	76·8	76·7
1·0	86·5	86·2

Intermediate values may be obtained by interpolation.

B = observed barometric pressure.

Normal range—adult 53–77 c.c., infants about 10 per cent. lower.

71. Blood Pressure Model.

(*a*) *General distribution.* Examine the schema (Fig. 114) of the circulation given you and identify the parts representing arteries, capillaries and veins. Disconnect the rubber ball *H* and the two Bunsen valves *V*, *V*. Attach the arterial tube *A* to the water supply and lead the tube from *G* to the sink. Cautiously turn on the water and measure the pressure in the arteries (at *B*), and in the veins (at *E*). (It is more economical to have single vertical tubes at *B* and *E*, the pressures read off in millimetres of water may be calculated in millimetres of mercury.)

Note the effect of (*a*) varying the force of inflow by manipulation of the water tap, (*b*) varying the resistance to flow by tightening the clip at *D*. With a steady pressure over its whole length, compress *G* and note alteration in manometer levels.

(*b*) *Pulse.* Fill with water and replace *H* and *V–V* in circuit. Gently compress and relax *H* at regular rhythmic intervals of about a second. Note

the effect of this upon the arterial and venous pressures. Study the further effect of constricting *D*.

(*c*) Place a finger on *A* and note the expansion with each contraction of *H*. Study the same thing on *D* and on *F*.

72. **Vowel Sounds by Percussion.**

Place the mouth in the position necessary for the pronunciation of the various vowels and then percuss over the cheek (Fig. 96). Now shift the point of percussion to a position over the pharynx just behind the angle of the jaw and percuss again. Note that the "cheek notes" rise as one passes from U—O—A—E—I, while the "pharynx notes" rise U—O—A and fall to E and I. This demonstrates the double nature of the mouth cavity in producing E and I (Fig. 97).

73. **Prepare a series of bladders** filled completely with (*a*) air, (*b*) water and (*c*) some viscous fluid like syrup and (*d*) lard. Percuss and palpate each.

74. **Demonstration of the Effect of Colour on the Absorption of Radiant Energy** (L. Hill).

Two similar pieces of cotton tape, one white and the other black, are

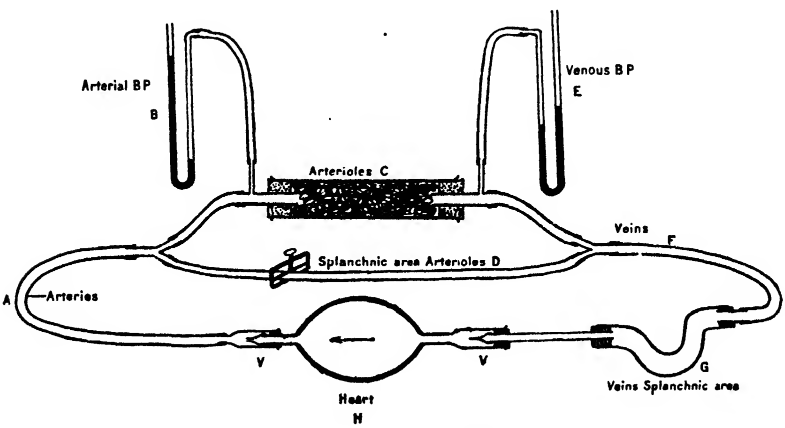


FIG. 114.—Schema of the circulation.

suspended so that the end of each dips in a graduated cylinder filled with water. Place first in the shade and measure the amount of water evaporated from each cylinder during a period of 30 minutes. Refill with water to the same level and repeat the experiment in direct sunlight.

75. **The Kata-thermometer.**

The instrument is fully described in Chap. XXXII. Before attempting to use it the student should study the description and the theory outlined there.

Cautions. (i.) The bulb is very fragile, and should be handled carefully. With reasonable use the kata-thermometer should have a life as long as an ordinary thermometer and longer than a clinical thermometer.

(ii.) The kata-thermometer should *never* be put into boiling water and should *never be left without attention* in warm water.

Method. (*a*) See that there is a *continuous* thread of spirit in the stem reaching from bulb to upper reservoir (see text, Chap. XXXII.). Dry the bulb.

(*b*) Hang the instrument by a thin cord to a stand (a wooden filter stand answers well) and start a stop-watch when the meniscus reaches the upper

mark. Stop the watch when the meniscus reaches the lower mark. Note the interval of time.

(*c*) Repeat the experiment, warming the bulb as in (*a*).

(*d*) Repeat as in (*c*).

If the readings are reasonably consistent average them and calculate the heat loss as in Chap. XXXII.

(*e*) Put on the silk thimble and repeat (*a*), (*b*), (*c*) and (*d*). See text.

PREPARATIONS

76. Water for Faraday-Tyndall Phenomenon.

Store a large volume of glass-distilled water in a paraffin covered jar stoppered with a paraffined cork through which pass two tubes, viz. a syphon delivery tube reaching only one-third of the way down inside the jar, and an air inlet tube protected by a plug of glass wool. The water should stand undisturbed for at least three months before use.

77. Collodions.

Cellulose nitrate (gun-cotton or pyroxylin) is generally sold damped with alcohol and, for very accurate work, should be dried before weighing. For the following experiments this refinement is unnecessary.

Acetic Acid Collodion. Four grams of commercial gun-cotton are placed in a wide-mouthed glass-stoppered bottle of 200 c.c. capacity; 100 c.c. glacial acetic acid are added. The mixture is shaken gently until the gun-cotton has dissolved, leaving no residue. The resulting sol is transparent and will keep for weeks.

Alcohol-Ether Collodion. (Bigelow and Gemberling.) 75 c.c. of ethyl ether are poured on 3 gm. of gun-cotton in a wide-mouthed stoppered bottle as above. After 10–15 minutes, 25 c.c. of ethyl alcohol are added and the mixture agitated until a clear solution is obtained.

Either of these collodion solutions (after standing till free from bubbles) may be used in the preparation of membranes.

The permeability of collodion membranes may be standardised for any particular make of collodion by standardising the conditions under which they are freed from ether and alcohol. Permeability can be *increased* by allowing the collodion membrane to set in a moist atmosphere or under a bell-jar whose content of air is saturated with alcohol, or, better still, acetone vapour, before immersion of the film in water. Another and more reliable method of attaining the same end is to add a less volatile constituent than ether and alcohol to the stock solution, and, during the pre-immersion period, evaporating completely the volatile solvents in a dry atmosphere. The formula for such a solution, due to Pierce, is as follows: Stock solution, 1 gm. nitrocellulose, x c.c. ethylene glycol, 25 c.c. absolute alcohol and enough anhydrous anæsthetic ether to make 100 c.c. The permeability is controlled by varying the amount (x c.c.) of the ethylene glycol from 0·5 c.c. for the least permeable membrane (about 0·02 c.c. of water per cm.^{2} per min.) to 15 c.c. for a membrane giving a filtration rate of about 0·5 c.c. per cm.^{2} per minute.

78. Preparation of Collodion Membranes.

Membranes are easily made in any size or shape, and, as they are transparent, are very convenient for general use. They are, as ordinarily prepared, unsatisfactory for the dialysis of whole blood or of bile.

(i.) *To cover a vessel similar to a Graham dialyser.* Cleanse a piece of plate glass thoroughly and polish it. Pour sufficient collodion sol on the centre of the plate to give a large enough membrane. *Avoid bubbles.* By

tilting the plate, spread the sol evenly over the surface of the plate and, if necessary, drain off any excess into the stock bottle, taking care to avoid any unevenness in the distribution of the collodion. The diameter of the sheet should be fully 2 in. larger than that of the dialysing glass. If an acetate sol has been used, immediately plunge the glass and adherent gel into cold water and leave it immersed for about half an hour to convert the acetate gel into a hydrogel. After the minimum time of immersion has elapsed, the collodion film may be readily detached from the glass, placed centrally over the rim of a dialysing cup, and fixed in place by a broad rubber band. The dialyser should now be tested for leaks by filling it with water and observing that no fluid escapes round the junction. The film must be kept moist or it will shrink and rupture.

The ether-alcohol gel is *not* placed in water at once like the above, but is allowed to dry in air or in alcohol vapour for a period depending on the permeability required. If a very permeable diaphragm is required, a glass trough is inverted over the film so as to prolong the period of gelation. Although the degree of drying is the crucial point of the whole process, no definite rules can be laid down. Each " make " of collodion requires treatment on its own merits and experience alone will tell when the film is ready. If the sol has been made as directed, it should be dry enough when it does not stick to the finger when touched *lightly*. The edges can be loosened with a spatula or paper knife and the whole film slowly lifted from the glass. When about three-fourths of the sheet has been raised vertically from the plate the rim of the dialysing cylinder is placed below it so that the edge of the rim comes in contact with the collodion surface which has been next the glass plate, *i.e.* with the surface which has been dried least. The edges of the membrane are carefully turned down over the sides of the cup and will adhere quite firmly. If desired, a broad rubber band may be placed round the rim to ensure tightness. Test, as above, for leaks and leave immersed in water for 10–15 minutes to allow the alcohol to be replaced by water.

(ii.) *To make a collodion sac.* A small Erlenmeyer flask, clean and dry, is filled with collodion sol, *care being taken to pour the fluid slowly down the side so as not to form air-bubbles.* The sol is now poured back into the stock. This should be done slowly and steadily with a constant rotatory motion of the flask, leaving a thin film adherent to the glass. If this operation is carried out too quickly, the layer of collodion at the bottom of the flask will be too thin. It is convenient to allow a little collodion to overflow all round the outside of the neck of the flask to enable one to get a good grip in pulling out the film afterwards. The flask (acetate sols are at this stage submersed in water) is inverted over the mouth of a bottle containing a little alcohol (or empty, see above), and allowed to dry as before. When dry, the flask is filled with water, or, better still, immersed in water and allowed to stand for at least 15 minutes. The collodion sac is loosened at the neck and carefully withdrawn. This is a slow process and must not be hurried or grave risk will be run of tearing the thin film. It is quite a good thing to remove the film in stages, letting it soak in water between times. The complete bag is usually fitted with a glass mouth—a piece of glass tubing of suitable size inserted in the neck and affixed thereto by broad rubber bands, or by wrapping in oiled silk, gutta-percha tissue, etc., and winding thin string over this, or by causing it to adhere with a little fresh collodion.

(iii.) *To make collodion test tubes.* (*a*) Collodion tubes may be formed

inside test tubes or boiling tubes in the same manner as has been described for sacs.

(*b*) Sometimes it is desirable to make a dialysing tube which will fit on the *outside* of a specified tube. A test tube of the required width is taken and a small hole blown in the end. This hole is covered with a thin film of collodion which is allowed to dry. The whole tube is then dipped in the collodion solution. The excess collodion is allowed to drip into stock, the test tube being held bottom upwards at an angle of less than 45 degrees and rotated steadily. The film is treated with water, or after drying as above, depending on the nature of the solution. After soaking in water, the test tube is filled with water and the film gently worked off like a tight glove. It is eased a little at the bottom, water meanwhile passing into the collodion tube through the hole in the test tube. Little by little a fine film of moisture will creep up between film and glass and the transparent collodion tube will slip off easily. This method yields tubes which are more uniform in thickness and permeability than the former.

(iv.) *Thimbles.* Strong dialysing vessels may be made by impregnating Soxhlet extraction thimbles with collodion. The thimble, soaked in alcohol, is immersed in alcohol-ether collodion, withdrawn, allowed to drip and partially dried, and then the process of impregnation is repeated. It is advisable to fix a short glass tube into the mouth of the thimble before impregnation and to use a fairly thin collodion solution.

79. **Parchment Dialysers.**

Parchment is sold for this purpose in sheets, or made up in long tubes (sausage skins) or in thimbles. When dry the paper becomes very brittle, so that great care has to be observed in its storage to prevent creases, etc.

(i.) *Sheet.* Select a piece of the paper free from obvious defects (pin-holes, etc.) and fully 2 in. larger in each direction than is required. Soak in water till soft and pliable. Place it centrally over a dialysing glass as is described above for collodion sheets. The folding of the free edges requires some care to ensure even lying against the side of the glass. Tie with thin string. Test for leaks.

The sheet may be made into a bag as follows: "Cut a regular hexagon and soak it thoroughly in water. Then place it centrally on the bottom of an inverted beaker or jar, the diameter of which is about one-third of that of the inscribed circle of the hexagon. Gently pinch radial folds from the circumference of the beaker to the corners of the hexagon and mould them so that the paper midway between the corners touches the wall of the beaker, and then turn the folded portions over and smooth them into cylindrical shape. The folds must not be sharp, as even wet parchment may be damaged by too drastic treatment. When the bag has been moulded as described, a string is loosely tied around it, or a fairly slack rubber band slipped over it within about 2 in. of the edge, and the bag is then drawn off the beaker. Its permanent shape is secured by threading a clean thin string through the folds, which is gently drawn tight after every completed stitch so that the circumference at the open end is roughly the same as at the bottom. The bag is suspended in a jar of suitable size by two or three strings tied at equal distances to the string which secures the circumference." (From Hatschek's *Laboratory Manual*, Messrs. Churchill.)

(ii.) Similar care must be taken when working with parchment tubes or thimbles. The "*sausage-skin dialysers*" are excellent for demonstration purposes, as they offer a large effective surface. They are sold flat in lengths of about a metre and are very easily damaged. They are best kept hanging

from one end. Thoroughly soak and test a piece. Cut it to a convenient length and with a large cork-borer excise a circular piece from both ends about ½ in. from the opening. Bend the tube into U-shape and place it in a tall cylinder. A glass rod longer than the diameter of the cylinder thrust through the holes at the ends of the dialyser acts as support. The tube may now be filled, by means of a funnel, with the fluid to be dialysed, while the cylinder is filled with water *at the same time and at the same rate.* This is to prevent undue strain on the tube.

TYPICAL COLLOIDS

(a) SOLS

80. **Preparation of Colloidal Gold.**

(1) *Protected Solution* (Ostwald). To 100 c.c. of ordinary distilled water add a few drops of 1 per cent. neutral gold chloride. Mix and add a few drops of 0·1 per cent. tannic acid sol. Heat over a free flame till boiling, shaking it constantly. If the red colour does not appear on boiling add a little more tannic acid and a little more gold chloride alternately. Divide into two parts.

A. To one part, while still hot, add about an equal volume of water.

B. Cool the other part before diluting. *A* is violet in colour while *B* is cherry red. Blue gold sol. may be prepared from neutral 0·05 gold chloride. Take three portions of the gold chloride solution and add (i.) 5–10 drops, (ii.) 10–15 drops, and (iii.) 15–20 drops respectively of a hydrazine hydrochloride solution prepared by adding a tiny crystal to about 20 c.c. of water. If (ii.) does not turn blue add more hydrazine. If it is greenish, too much hydrazine has been added.

(2) *Gold Sol for Colloidal Gold Test.* Heat 150 c.c. of triply distilled water (from a resistance glass still). Add 1 c.c. of 1 per cent. gold chloride solution and then 2·5–3 c.c. N/5 pure K_2CO_3. Bring to the boil, stirring vigorously. Add gradually, but not too slowly, 2–3 c.c. of 1 per cent. commercial formalin (1 c.c. 40 per cent. formalin in 99 c.c. of water) and remove the flame. See that the sol (which should be ruby red without any purple tinge) is neutral to alizarin red and that a 5 c.c. sample of it is completely reduced in one hour by adding 1·7 c.c. of 1 per cent. NaCl.

(3) *Determination of the C_H of Colloidal Gold.* Take a series of small test tubes of equal bore, etc., and range them in two equal rows. Into each tube put 1 c.c. of triply distilled water. To the first tube of one row add 1 c.c. of N/50HCl. Mix. Remove 1 c.c. of the mixture and add this to the second tube, and so on down the row. This will give a series of acid solutions as follows : N/100, N/200, etc. The other series of tubes is treated in exactly the same way with N/50NaOH. The final 1 c.c. of each series is discarded. Two drops of alizarin red and 1 c.c. of the gold sol are added to each tube and the contents mixed. The tube showing the neutral alizarin colour is picked out and its value determined. The whole gold sol is now neutralised by the addition of the exact amount of NaOH or HCl as the case may be.

81. **Colloidal Iron** (Experiment 26 (*d*)).

Heat 250 c.c. of distilled water in a 500 c.c. Erlenmeyer flask or in a tall beaker flask. When the water is boiling vigorously, place the burner a little out from the centre of the flask so as to produce a rotatory movement of the water. Add to the boiling water, drop by drop, 1 c.c. of a 30 per cent. solution of ferric chloride. The liquid turns a clear reddish brown colour,

The sol contains 2·2 millimoles per cent. of hydrochloric acid from the 0·3 gm. of $FeCl_3$. Most of this acid can be removed by dialysis. *Caution.* Do not push dialysis too far or the sol will undergo coagulation. The undialysed sol may be used for the experiments on cataphoresis, diffusion, precipitation and protection. The dialysed sol or B.P. dialysed iron are necessary for the experiment on mutual precipitation.

82. **Sulphur Sol.**

(i.) *Odén's.* Prepare 100 c.c. of 2N (approx.) sulphurous acid by titration against phenolphthalein. Pass clean H_2S for an hour or so through it and continue till the smell of SO_2 has practically disappeared. Allow the fluid to stand for 24 hours and then decant off the milky yellow supernatant sol. Before use dilute the sol 100 times with distilled water.

(ii.) A sulphur sol may be prepared by neutralising a very dilute solution of ammonium sulphide with hydrochloric acid.

83. **Purple of Cassius.**

Add a few cubic centimetres of a 0·01 per cent. solution of *stannous chloride* to a 0·05 per cent. solution of gold chloride. The stannous chloride reduces the gold chloride (cf. tannic acid and formalin), producing colloidal gold, and itself being converted in the process to the stannic form. The colloidal *stannic acid* formed from this is a hydrophilic colloid and "protects" the gold. The purple-red colour is, therefore, due to an adsorption complex of gold and tin.

84. **Gelatin 1 per cent. Sol.**

(*a*) Free the gelatin from the bulk of extraneous matter by immersing it in a large bulk (about 100 vols.) of cold distilled water. Change the water several times.

(*b*) Boil about 75 c.c. of distilled water for every 100 c.c. of sol required. Remove the flame and add the cleaned and *swollen* gelatin as free as possible from wash water. Stir continuously till the gelatin has completely dissolved. Cool.

(*c*) When quite cold stir in the amount of distilled water necessary to give 100 c.c. or whatever multiple of 100 c.c. is desired. Accuracy may be obtained by first weighing the beaker, then beaker plus cooled contents, and so arriving at the weight of additional water necessary.

85. **Starch 1 per cent.**

(*a*) Put the weighed potato starch in a mortar, and, adding small quantities of cold water at a time, grind into a smooth thick cream.

(*b*) Gradually stir in about half of the total volume of water required to make a 1 per cent. sol. This water should be fairly warm, but *not hot.*

(*c*) Dilute to about twice its volume with warm water.

(*d*) Boil on a sand bath for half an hour to convert dispersoid to a true colloid.

(*e*) Cool, and *when cold* make up to the desired volume with cold water.

(*f*) Filter through a well washed grey paper.

(*g*) Cover the surface with a *thin* film of toluene to prevent rapid bacterial infection.

86. **Gum Mastic.**

One to five grams of gum mastic are placed in a medium-sized mortar and dissolved by trituration with repeated amounts of 96 per cent. alcohol. The solution is made up to 100 c.c. with alcohol and filtered; 10 c.c. of this solution are placed in a 500 c.c. beaker and 200 c.c. of distilled water added at once in one portion. Filter and allow to stand for about an hour. Use the upper two-thirds of the suspensoid.

87. Silicic Acid Sol from Water-glass.

Dilute some "water-glass," "keep-egg" or other commercial preparation of the same nature with enough *freshly boiled* (CO_2 free) distilled water to reduce its specific gravity to 1·16. This stock solution can be kept for years in a wide-mouthed bottle closed by a rubber bung or a well-vaselined glass stopper.

To prepare a sol (Hatschek) dilute 30 c.c. of concentrated hydrochloric acid (1·2 specific gravity) with 100 c.c. of water, and pour 75 c.c. of the stock sodium silicate solution *into the dilute acid.* Dialyse the mixture in a parchment bag against repeated changes of water. *Caution. Do not push the dialysis too far or the sol will set to a gel.*

The sol should be perfectly clear and colourless, and absolutely free of any bluish tinge (which is indicative of impending gelation). The sol may be kept in wide-mouthed bottles as above. *It is very sensitive to* CO_2, *either free or bound.*

88. **Coarse Suspensions.**

Gamboge, Indian Ink, Chinese Ink, etc. A small quantity (1–2 gm.) of the block is placed in a mortar and ground with a few cubic centimetres of water. The resulting mixture is diluted with a large volume (500–1,000 c.c.) of water. The coarser particles are removed by filtration.

89. (i.) **Egg White Solution (from Eggs).**

(*a*) Separate white from yolk.

(*b*) Beat white to a foam and allow to stand.

(*c*) Remove membranous foam from the clear fluid below.

(*d*) Add 0·9 per cent. solution of sodium chloride to make the volume five times the volume of (*c*).

(*e*) Add 300 c.c. of water slowly, stirring regularly.

(ii.) *Egg Albumin Sol* (*from commercial dried egg albumin*). Put 10–15 gm. of the commercial albumin ex ovis crushed in a coffee mill or large mortar into 100 c.c. of water in a bottle and leave overnight to allow of imbibition. Add 5 drops of 1 per cent *thymol* in chloroform and put the bottle on a mechanical shaker to break up most of the larger lumps. Leave standing a few hours to let the larger lumps settle. Filter through a large glass funnel with a short stem into which has been placed a plug of cotton-wool. This filtration will require twelve hours or more, but should yield a yellowish opalescent fluid.

A more economical but more tedious method is to add the dried egg white to the water in small portions, stirring regularly and attempting to break up the lumps with the glass rod.

90. **Finely Divided Suspension of Protein for Use in Experiments on Proteolytic Enzymes.**

(i.) Take a measured quantity of sheep's blood serum and dilute it with 15 volumes of distilled water. Add 10 per cent. sulpho-salicylic acid solution until the mixture imparts a faint violet tint (*not blue*) to Congo red paper. At this point the diluted serum appears a homogeneous white due to the fine permanent suspension of protein produced.

(ii.) Milk, centrifuged and filtered from fatty particles through *wet* paper is a very sensitive substrate for proteolytic enzymes.

(b) GELS

91. **Egg Albumin.**

(i.) If at (*e*) in Experiment 89 (i.) the 300 c.c. of water added to the sol were *boiling*, a fine suspension of coagulated egg white would result.

(ii.) *For Mett's tubes.* Suck up some of sol 89 (*d*) or undiluted blood serum stained with methylene blue or gentian violet into capillary tubes (about 2 mm. bore). See that the aspirated sol is free from air bubbles. Lay the tubes horizontally on a piece of gauze, wrap them and tie the gauze into a bag. Immerse the tubes in water (which has been boiled and allowed to cool to 85° C.) for 2–3 minutes. Cool and dry the tubes on filter paper. Dip the extreme ends in melted paraffin to seal the tubes. When required cut the tubes into lengths of about 1 cm., discarding about 1 cm. from each end.

92. **Concentrated Gelatin.**

Sheets of Coignets "Gelatin A" washed for 48 hours in three changes of ice-cold water acidified to pH 4·6 originally (falling to 4·9 eventually). Warm (below 60° C.), and it dissolves in its own water of imbibition. Keep liquid under 40 c.c. Add some toluene as preservative.

93. **Non-polarisable Electrodes.**

Brush electrodes of Fleischl (for Experiments 54, 55, etc.). The electrodes consist of glass tubes (4 cm. × 5 mm.). In one end fits a perfectly clean camel's-hair pencil, and into the other dips a well-amalgamated rod of zinc with a binding screw at its free end. A piece of indiarubber tubing fits as a cap over the upper end of the tube, holding the zinc rod in position.

Preparation of the electrode. Make a paste of kaolin, using normal saline solution as the moistening agent. Pack the paste into the tube to cover the fixed end of the brush and to give a layer of about ½ cm. in thickness.

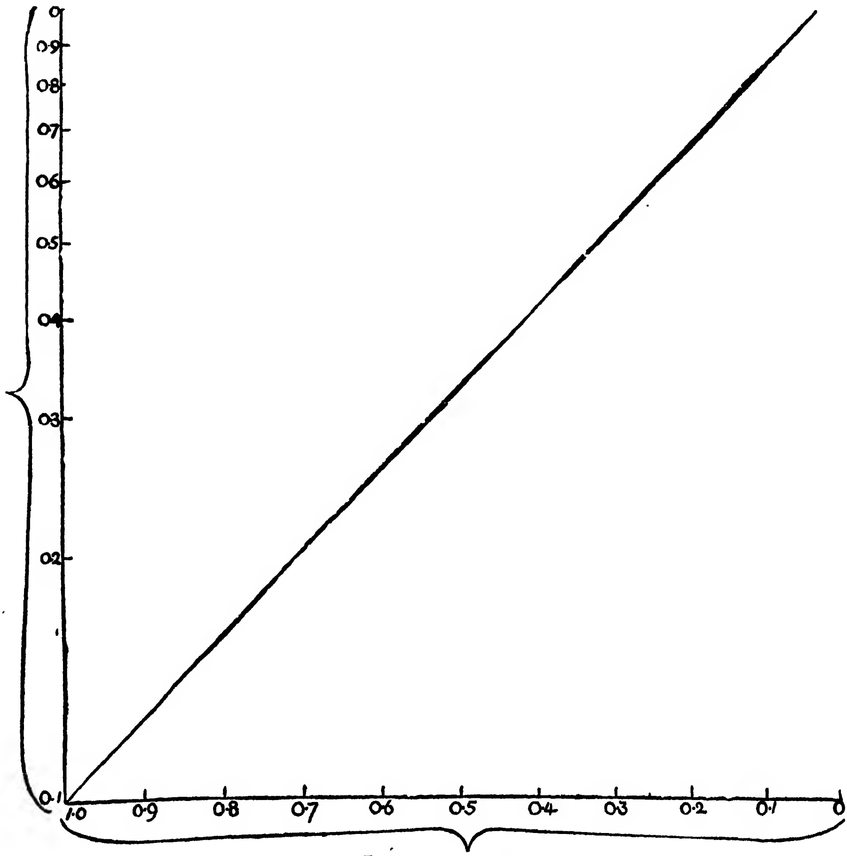
(At this stage in the preparation the electrodes are placed in normal saline solution until required for use.)

When required the tubes are filled with a saturated solution of $ZnSO_4$ and the zinc rods are inserted.

CONVERSION FACTORS

94. Graphic Conversion of Sørensen's pH($-\log$ (H·)) **into Concentrations of Hydrogen Ions and the Reverse** (Roaf, *Journ. of Physiol.*).

Fig. 115 is a graph whereby the logarithmic notation of Sørensen can be converted at sight into true concentrations. It would be advisable to redraw the figure on semi-logarithmic paper and so enable the reading to be taken



[H·] or $C_H = 10^{-x}$ $-\log$ [H·] or pH $= x + \ldots$

FIG. 115.—Graph for conversion of pH to C_H.

to two places of decimals For example, to convert pH6·7 into C_H, pH $= 6$, *i.e.* $C_H = 10^{-6}$; 0·7 cuts the diagonal line opposite 0·2; therefore

$$p\text{H of } 6{\cdot}7 = C_H \text{ of } 0{\cdot}2 \times 10^{-6}.$$

95. Estimation of the Surface Area of the Body.

Rubner announced that *the amount of heat produced by an animal is proportional to its superficial area.* This law of surface area has rendered necessary the accurate determination of the skin surface in most metabolism experiments.

Since the *surface* of a figure varies as the square, and that of *volume* as the cube of its linear dimensions, it follows that

$$S = K\sqrt[3]{V^2},$$

where S = surface, V = volume and K = constant, or, as weight (W) varies directly as volume,

$$S = K\sqrt[3]{W^2}.$$

Meeh, from sixteen experiments, suggested that K should be = 12·3 for adults; and Lissauer used the value 10·3 for children. The average error of this calculation is about 16 per cent.

The brothers Du Bois covered the body-surface of their subjects with "tights" and applied melted paraffin. Paper strips were affixed to prevent change of area during the process of removing the "shell." The model of the surface, cut into pieces sufficiently small to be flat, was photographed upon

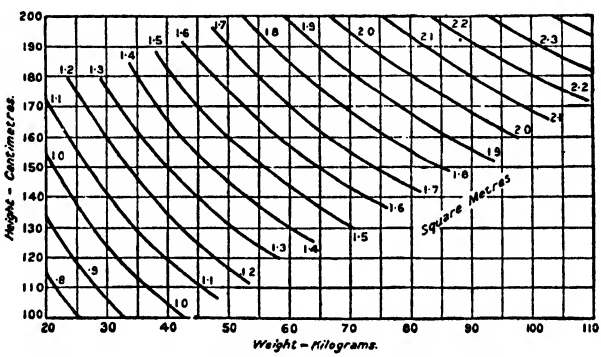


FIG. 116.—Du Bois graphs for estimating the area of body-surface.

squared paper of uniform thickness. The weight of each square on the paper was known. The darkened portions of the paper were carefully cut and weighed, and from this was calculated the area of body surface. The formula resulting from this work involved nineteen measurements. From this they have evolved a two-measurement formula on which the appended chart is based (Fig. 116).

Sundry Conversion Factors

		Multiply by
Length . .	Inches to centimetres	2·54.
Area . .	Square inches to square centimetres .	6·4516.
Volume . .	Cubic inches to cubic centimetres . .	16·387.
	Gallons to litres	4·546.
Weight . .	Ounces to grams	28·349.
	Grains to grams	0·0648.
	Pounds (avoirdupois) to kilos . . .	0·4536.
Force . .	Poundals to dynes	13,825.
	Pounds weight to dynes	$4{\cdot}45 \times 10^5$.
Velocity . .	Miles per hour to centimetres per second .	44·70.

Pressure . .	Pounds per square inch to dynes per square centimetre	68,971.
	Atmospheres to pounds per square inch .	14·7.
	Atmospheres to dynes per square centimetre	$1{\cdot}0132 \times 10^6$.
	Head of water in feet to pounds per square inch	0·43.
Density . .	Pounds per cubic foot to grams per cubic centimetre	0·01602.
Energy . .	Joules to ergs (1 erg = 1 dyne per second)	10^7.
	Gram-calories to joules . . .	4·19.
	Gram-calories to kilogram-metres . .	0·4266.
	Foot-pounds to kilogram-metres . .	0·1382.
	Foot-pounds to joules	1·356.
Power . .	(1 watt = 1 joule per second = 1 volt-ampère.)	
	Watts to kilogram-metres per second .	0·102.
	Horse-power to watts	746.
	Horse-power to kilogram-metres per second	76·04.

1 B.T.U. = power of 1 kilowatt for one hour.
= $3{\cdot}6 \times 10^6$ joules.
= $8{\cdot}59 \times 10^5$ gram-calories.

German candles to English candles . . 1·25.

Thermometers. To convert degrees F. into degrees C., deduct 32 and multiply by 5/9. To convert degrees C. into degrees F., multiply by 9/5 and add 32.

1 litre of oxygen at 0° C. and 760 mm. weighs 1·429 grams.
1 litre of carbon-dioxide at 0° C. and 760 mm. weighs 1·965 grams.
1 litre of nitrogen at 0° C. and 760 mm. weighs 1·254 grams.
1 litre of air at 0° C. and 760 mm. weighs 1·292 grams.
1 litre of hydrogen at 0° C. and 760 mm. weighs 0·900 gram.
1 litre of water vapour at 0° C. and 760 mm. weighs 0·8132 gram.
1 gram of protein ≡ 966·3 c.c. oxygen intake and 773·9 c.c. of carbon-dioxide output.
1 gram of fat ≡ 2019·3 c.c. oxygen intake and 1427·3 c.c. of carbon-dioxide output.
1 gram of starch ≡ 828·8 c.c. oxygen intake and 828·8 c.c. of carbon-dioxide output.
1 gram of urinary nitrogen ≡ 26·51 Calories.
≡ 5·91 litres (8·45 grams) of oxygen.
≡ 4·75 litres (9·35 grams) of carbon-dioxide.

1μ = 1 micromillimetre = 10^{-3} mm.
$1\mu\mu$ = 1 millimicrometre = 10^{-6} mm.
1 Å = 1 Ångström unit = $0{\cdot}1\ \mu\mu$ = 10^{-7} mm.

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CATHCART. In " Practical Physiology " (*ibid.*).

Histology.

WATSON. " Handbook of Histology." Livingstone.

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